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(54) Title: ANTAGONISTS

(57) Abstract: Peptide sequences capable of binding to insulin and/or insulin-like growth factor receptors with either agonist or antagonist activity and identified from various peptide libraries are disclosed. This invention also identifies at least two different binding sites, which are present on insulin and insulin-like growth factor receptors, and which selectively bind the peptides of this invention. As agonists, the peptides of this invention may be useful for development as therapeutics to supplement or replace endogenous peptide hormones. The antagonist peptides may also be developed as therapeutics.



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INSULIN AND IGF-1 RECEPTOR AGONISTS AND ANTAGONISTS

This application is a continuation-in-part of U.S. Application Serial No. 09/962,756 filed September 24, 2001, which is a continuation-in-part of U.S. Application Serial No. 09/538,038 filed March 29, 2000, which is a continuation-in-part of U.S. Application Serial No. 09/146,127, filed September 2, 1998, all of which are incorporated herein by reference in their entirety.

I. FIELD OF THE INVENTION

This invention relates to the field of hormone receptor activation or inhibition. More specifically, this invention relates to the identification of molecular structures, especially peptides, which are capable of acting at either the insulin or insulin-like growth factor receptors as agonists or antagonists. Also related to this invention is the field of molecular modeling whereby useful molecular models are derived from known structures.

II. BACKGROUND OF THE INVENTION

Insulin is a potent metabolic and growth promoting hormone that acts on cells to stimulate glucose, protein, and lipid metabolism, as well as RNA and DNA synthesis. A well-known effect of insulin is the regulation of glucose levels in the body. This effect occurs predominantly in liver, fat, and muscle tissue. In the liver, insulin stimulates glucose incorporation into glycogen and inhibits the production of glucose. In muscle and fat tissue, insulin stimulates glucose uptake, storage, and metabolism. Defects in glucose utilization are very common in the population, giving rise to diabetes.

Insulin initiates signal transduction in target cells by binding to a specific cell-surface receptor, the insulin receptor (IR). The binding leads to conformational changes in the extracellular domain of IR, which are

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transmitted across the cell membrane and result in activation of the receptor's tyrosine kinase activity. This, in turn, leads to autophosphorylation of tyrosine kinase of IR, and the binding of soluble effector molecules that contain SH2 domains such as phosphoinositol-3-kinase, Ras GTPase-activating protein, and phospholipase C γ to IR (Lee and Pilch, 1994, *Am. J. Physiol.* **266**:C319-C334).

Insulin-like growth factor 1 (IGF-1) is a small, single-chain protein (MW = 7,500 Da) that is involved in many aspects of tissue growth and repair. It is similar in size, sequence, and structure to insulin, but has 100-1,000-fold lower affinity for IR (Mynarcik *et al.*, 1997, *J. Biol. Chem.* **272**:18650-18655). Although IGF-1 mRNA can be detected in many tissues, the majority of circulating IGF-1 is produced in the liver after stimulation by growth hormone (Butt *et al.*, 1999, *Immunol. Cell Biol.* **77**:256-262). Functionally, IGF-1 appears to act as a mitogen and as an anti-apoptotic factor for cells.

Recent studies have analyzed the role of endogenous IGF-1 in various disease states. Several reports have shown that IGF-1 promotes the growth of normal and cancerous prostate cells both *in vitro* and *in vivo* (Angelloz-Nicoud and Binoux, 1995, *Endocrinol.* **136**:5485-5492; Figueroa *et al.*, 1995, *J. Clin. Endocrinol. Metab.* **80**:3476-3482; Topping *et al.*, 1997, *J. Urol.* **158**:222-227). Elevated serum levels of IGF-1 have been shown to be associated with increased risks of prostate cancer, and may be an earlier predictor of onset than prostate-specific antigen (PSA; J.M. Chan *et al.*, 1998, *Science* **279**:563-566). Serum levels of free IGF-1 are regulated by the presence of IGF binding proteins (IGFBP), which bind to IGF-1 and prevent its interaction with the IGF-1R (reviewed in C.A. Conover, 1996, *Endocr. J.* **43S**:S43-S48; Rajaram *et al.*, 1997, *Endocr. Rev.* **18**:801-831). PSA has been shown to be a protease that cleaves IGFBP-3, resulting in an increase of free IGF-1 in serum (P. Cohen *et al.*, 1992, *J. Clin. Endocrinol. Metab.* **75**:1046-1053; P. Cohen *et al.*, 1994, *J. Endocrinol.* **142**:407-415; H. Lilja, 1995, *Scand. J. Clin. Lab. Invest. Suppl.* **220**:47-56). Consistent with

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this finding, men with higher levels of circulating IGF-1 and lower levels of IGFBP-3 were found to be at higher risk for developing colorectal cancer (J. Ma *et al.*, 1999, *J. Natl. Cancer Instit.* **91**:620-625.). Recent studies have also shown a connection between IGF-1 levels and ovarian cancer.

5 There also appears to be a relationship between high levels of IGF-1 and/or IGF-1R and breast cancer (L.C. Happerfield *et al.*, 1997, *J. Pathol.* **183**:412-417). A positive correlation was observed between circulating IGF-1 and breast cancer among pre-menopausal women (S.E. Hankinson *et al.*, 1998, *Lancet* **351**:1393-1396). A poor prognosis for breast cancer patients
10 was correlated to the expression of IGF-1R positive and estrogen receptor (ER) negative cells (A.A. Butler *et al.*, 1998, *Cancer Res.* **58**:3021-3027). Recently, investigators have identified hybrid IGF-1R/IR receptors found in several breast cancer cell lines (G. Pandini *et al.*, 1999, *Clin. Cancer Res.* **5**:1935-1944; E.M. Bailyes *et al.*, 1997, *Biochem. J.* **327**(Pt 1):209-215; see
15 below). The data has suggested that these hybrids behave as functional IGF-1Rs and may play a major role in IGF-1 signaling in breast cancer.

Clinical studies have also investigated the use of recombinant human IGF-1 in the treatment of several diseases, including type I diabetes (Carroll *et al.*, 1997, *Diabetes* **46**:1453-1458; Crowne *et al.*, 1998, *Metabolism*
20 **47**:31-38), amyotrophic lateral sclerosis (Lai *et al.*, 1997, *Neurology* **49**:1621-1630), and diabetic motor neuropathy (Apfel and Kessler, 1996, *CIBA Found. Symp.* **196**:98-108). Other potential therapeutic applications of IGF-1, such as osteoporosis (Canalis, 1997, *Bone* **21**:215-216), immune modulation (Clark, 1997, *Endocr. Rev.* **18**:157-179) and nephrotic syndrome
25 (Feld and Hirshberg, 1996, *Pediatr. Nephrol.* **10**:355-358) are also under investigation. Clearly, IGF-1R activity is involved in many disease states, indicating that there are potential clinical applications for both IGF-1 agonists and antagonists.

Both insulin and IGF-1 are expressed as precursor proteins
30 comprising, among other regions, contiguous A, B, and C peptide regions, with the C peptide being an intervening peptide connecting the A and B

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peptides. A mature insulin molecule is composed of the A and B chains connected by disulfide bonds, where the connecting C peptide has been removed during post-translational processing. IGF-1 retains its smaller C-peptide as well as a small D extension at the C-terminal end of the A chain, making the mature IGF-1 slightly larger than insulin (Blakesley, 1996). The C region of human IGF-1 appears to be required for high affinity binding to IGF-1R (Pietrzkowski *et al.*, 1992, *Cancer Res.* **52**(23):6447-51). Specifically, tyrosine 31 located within this region appears to be essential for high affinity binding. Furthermore, deletion of the D domain of IGF-1 increased the affinity of the mutant IGF-1 for binding to the IR, while decreasing its affinity for the IGF-1R (Pietrzkowski *et al.*, 1992). A further distinction between the two hormones is that, unlike insulin, IGF-1 has very weak self-association and does not hexamerize (De Meyts, 1994).

IGF-1 and insulin competitively cross-react with IGF-1R and IR (L. Schäffer, 1994, *Eur. J. Biochem.* **221**:1127-1132). Yet, despite 45% overall amino acid identity, insulin and IGF-1 bind only weakly to each other's receptor. The affinity of each peptide for the non-cognate receptor is about 3 orders of magnitude lower than that for the cognate receptor (Mynarcik, *et al.*, 1997, *J. Biol. Chem.* **272**:18650-18655). The differences in binding affinities may be partly explained by the differences in amino acids and unique domains which contribute to unique tertiary structures of ligands (Blakesley *et al.*, 1996, *Cytokine Growth Factor Rev.* **7**(2):153-9).

IGF-1R and IR are related members of the tyrosine-kinase receptor superfamily of growth factor receptors. Another family member is insulin-related receptor (IRR), for which no natural ligand is known. Both IGF-1R and IR are comprised of two α and two β subunits which form a disulfide-linked heterotetramer (β - α - α - β). These receptors have an extracellular ligand binding domain, a single transmembrane domain, and a cytoplasmic domain displaying the tyrosine kinase activity. The extracellular domain is composed of the entire α subunits and a portion of the N-terminus of the β

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subunits, while the intracellular portion of the β subunits contains the tyrosine kinase domain. In contrast to other tyrosine kinase receptors, IGF-1R, IR and IRR exist on the cell surface as disulphide-linked dimers and require domain rearrangements rather than receptor oligomerization for cell signaling (Adams *et al.*, 2000, *Cell. Mol. Life Sci.* **57**:1050-1093; Garrett *et al.*, 1998, *Nature* **394**:395-399; Frasca *et al.*, 1999, *Mol. Cell Biol.* **19**: 3278-3288; De Meyts *et al.*, 1994, *Hormone Res.* **42**:152-169). In addition, insulin and IGF-1 hemireceptors (comprising one α subunit and one β subunit) can heterodimerize to form IR/IGF-1R hybrids (M.A. Soos *et al.*, 1990, *Biochem. J.* **270**:383-390; J. Kasua *et al.*, 1993, *Biochemistry* **32**:13531-13536; B.L. Seely *et al.*, 1995, *Endocrinology* **136**:1635-1641).

In many cells, IR/IGF-1R hybrids are the most common receptor subtype (Baillies *et al.*, 1997, *Biochem. J.* **327**(pt.1):209-215). The proportion of total IGF-1R assembled into hybrids varies between 40% and 60% in human tissues (M. Federici *et al.*, 1997, *Mol. Cell. Endocrin.* **129**(2):121-6). IR/IGF-1R hybrids are also overproduced in human cancer cells as a result of overexpression of IR and IGF-1R (Pandini *et al.*, 1999, *Clin. Cancer Res.* **5**:1935-1944; A. Belfiore *et al.*, 1999, *Biochemie*, **81**(4):403-7; V. Papa *et al.*, 1990, *J. Clin. Invest.* **86**:1503-1510; V. Papa *et al.*, 1993, *Cancer Res.* **53**:3736-3740). In particular, increased levels of IR/IGF-1R hybrids have been observed in breast cancer cell lines and breast cancer tissue specimens (Pandini *et al.*, 1999, *Clin. Cancer Res.* **5**:1935-1944). Similarly, high levels of IR/IGF-1R hybrids have been observed in thyroid cancer specimens and cell lines (A. Belfiore *et al.*, 1999, *Biochemie*, **81**(4):403-7). Functional studies have indicated that IR/IGF-1R hybrids are predominantly activated by IGF-1 (M.A. Soos *et al.*, 1993, *Biochem. J.* **290**(pt.2):419-426; A.L. Frattali *et al.*, 1993, *J. Biol. Chem.* **268**:7393-7400). Accordingly, it has been postulated that IR/IGF-1R hybrids provide additional binding sites for IGF-1, and thereby increase cell sensitivity to this factor (Baillies *et al.*, 1997, *Biochem. J.* **327**(pt.1):209-215;

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Pandini *et al.*, 1999, *Clin. Cancer Res.* **5**:1935-1944; A. Belfiore *et al.*, 1999, *Biochimie*, **81**(4):403-7).

IR is a glycoprotein having molecular weight of 350-400 kDa (depending of the level of glycosylation). It is synthesized as a single polypeptide chain and proteolytically cleaved to yield a disulfide-linked monomer α - β insulin receptor. Two α - β monomers are linked by disulfide bonds between the α -subunits to form a dimeric form of the receptor (β - α - α - β -type configuration). The α subunit is comprised of 723 amino acids, and it can be divided into two large homologous domains, L1 (amino acids 1-155) and L2 (amino acids 313-468), separated by a cysteine-rich region (amino acids 156-312) (Ward *et al.*, 1995, *Prot. Struct. Funct. Genet.* **22**:141-153). Many determinants of insulin binding seem to reside in the α -subunit. The β -subunit of IR has 620 amino acid residues and three domains: extracellular, transmembrane, and cytosolic. The extracellular domain is linked by disulfide bridges to the α -subunit. The cytosolic domain includes the tyrosine kinase domain, the three-dimensional structure of which has been solved (Hubbard *et al.*, 1994, *Nature* **372**:746-754). A unique feature of IR is that it is dimeric in the absence of ligand.

To aid in drug discovery efforts, a soluble form of a membrane-bound receptor was constructed by replacing the transmembrane domain and the intracellular domain of IR with constant domains from immunoglobulin Fc or λ subunits (Bass *et al.*, 1996, *J. Biol. Chem.* **271**:19367-19375). The recombinant gene was expressed in human embryonic kidney 293 cells. The expressed protein was a fully processed heterotetramer and the ability to bind insulin was similar to that of the full-length holoreceptor.

IGF-1R is synthesized as a 180 kDa precursor which is glycosylated, dimerized and proteolytically processed to yield mature receptor (T.E. Adams *et al.*, 2000, *Cell. Mol. Life Sci.*, **57**:1050-1093, 2000). The mature receptor/complex consists of two extracellular α -subunits and two transmembrane β -subunits having tyrosine kinase activity. IGF-1R is

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expressed in almost all normal adult tissue except for liver, which is itself the major site of IGF-1 production (Butt *et al.*, 1999, *Immunol. Cell Biol.* **77**:256-262). A variety of signaling pathways are activated following binding of IGF-1 to the IGF-1R, including Src and ras, as well as downstream pathways, such as the MAP kinase cascade and the PI3K/AKT axis (Chow *et al.*, 1998, *J. Biol. Chem.* **273**:4672-4680).

The sequence of IR is highly homologous to the sequence of IGF-1R, indicating that the three-dimensional structures of both receptors may be similar. The α -subunits, which contain the ligand binding region of IR and IGF-1R, exhibit between 47-67% overall amino acid identity. Three general domains, termed L1, cysteine-rich, and L2, have been reported for both receptors from sequence analysis of the α subunits. The cysteine residues in the cysteine-rich region are highly conserved between the two receptors; however, the cysteine-rich regions share only 48% overall amino acid identity. Notably, the crystal structure of the first three domains of IGF-1R has been determined (Garrett *et al.*, 1998, *Nature* **394**:395-399). The L domains consist of a single-stranded right-handed β -helix (a helical arrangement of β -strands), while the cysteine-rich region is composed of eight disulfide-bonded modules.

While similar in structure, IGF-1R and IR serve different physiological functions. IR is primarily involved in metabolic functions whereas IGF-1R mediates growth and differentiation. Consistent with this, ablation of IGF-1 (i.e., in IGF-1 knock-out mice) results in embryonic growth deficiency, impaired postnatal growth, and infertility. In addition, IGF-1R knock-out mice were only 45% of normal size and died of respiratory failure at birth (Liu *et al.*, 1993, *Cell* **75**:59-72). However, both insulin and IGF-1 can induce both mitogenic and metabolic effects. Whether each ligand elicits both activities via its own receptor, or whether insulin exerts its mitogenic effects through its weak affinity binding to IGF-1R, and IGF-1 its metabolic

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effects through IR, remains controversial (De Meyts, 1994, *Horm. Res.* **42**:152-169).

Also, despite the similarities observed between these two receptors, the role of the domains in specific ligand binding are distinct. Through
5 chimeric receptor studies, (domain swapping of the IR and IGF-1R α -subunits), researchers have reported that the sites of interaction of the ligands with their specific receptors differ (T. Kjeldsen *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* **88**:4404-4408; A.S. Andersen *et al.*, 1992, *J. Biol. Chem.* **267**:13681-13686). For example, the cysteine-rich domain of the
10 IGF-1R was determined to be essential for high-affinity IGF binding, but not insulin binding. When amino acids 191-290 of IGF-1R region was introduced into the corresponding region of the IR (amino acids 198-300), the modified IR bound both IGF-1 and insulin with high affinity. Conversely, when the corresponding region of the IR was introduced into the IGF-1R, the
15 modified IGF-1R bound to IR but not IGF-1.

A further distinction between the binding regions of the IR and IGF-1R is their differing dependence on the N-terminal and C-terminal regions. Both the N-terminal and C-terminal regions (located within the putative L1 and L2 domains) of the IR are important for high-affinity insulin binding but
20 appear to have little effect on IGF-1 binding for either IR or IGF-1R. Replacing residues in the N-terminus of IGF-1R (amino acids 1-62) with the corresponding residues of IR (amino acids 1-68) confers insulin-binding ability on IGF-1R. Within this region, residues Phe-39, Arg-41 and Pro-42 are reported as major contributors to the interaction with insulin (Williams *et al.*, 1995). When these residues are introduced into the equivalent site of
25 IGF-1R, the affinity for insulin is markedly increased, whereas, substitution of these residues by alanine in IR results in markedly decreased insulin affinity. Similarly, the region between amino acids 704-717 of the C-terminus of IR has been shown to play a major role in insulin specificity.
30 Substitution of these residues with alanine also disrupts insulin binding

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(Mynarcik *et al.*, 1996, *J. Biol. Chem.* **271**(5):2439-42; C. Kristensen *et al.*, 1999, *J. Biol. Chem.* **274**(52):37351-37356).

Alanine scans of IR and IGF-1R suggest that insulin and IGF-1 may use some common contacts to bind to IGF-1R but that those contacts differ from those that insulin utilizes to bind to IR (Mynarcik *et al.*, 1997). Hence, the data in the literature has led one commentator to state that even though "the binding interfaces for insulin and IGF-1 on their respective receptors may be homologous within this interface the side chains which make actual contact and determine specificity may be quite different between the two ligand-receptor systems" (De Meyts, 1994).

Based on data for binding of insulin and insulin analogs to various insulin receptor constructs, a binding model has been proposed. This model shows insulin receptor with two insulin binding sites that are positioned on two different surfaces of the receptor molecule, such that each alpha-subunit is involved in insulin binding. In this way, activation of the insulin receptor is believed to involve cross-connection of the alpha-subunits by insulin. A similar mechanism may operate for IGF-1R, but one of the receptor binding interactions appears to be different (Schäffer, 1994, *Eur. J. Biochem.* **221**:1127-1132).

The identification of molecular structures having a high degree of specificity for one or the other receptor is important to developing efficacious and safe therapeutics. For example, a molecule developed as an insulin agonist should have little or no IGF-1 activity in order to avoid the mitogenic activity of IGF-1 and a potential for facilitating neoplastic growth. It is therefore important to determine whether insulin and IGF-1 share common three-dimensional structures but which have sufficient differences to confer selectivity for their respective receptors. Similarly, it would be desirable to identify other molecular structures that mimic the active binding regions of insulin and/or IGF-1 and which impart selective agonist or antagonist activity.

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Although certain proteins are important drugs, their use as therapeutics presents several difficult problems, including the high cost of production and formulation, administration usually via injection and limited stability in the bloodstream. Therefore, replacing proteins, including insulin
5 or IGF-1, with small molecular weight drugs has received much attention. However, to date, none of these efforts has resulted in finding an effective drug replacement.

Peptides mimicking functions of protein hormones have been previously reported. Yanofsky *et al.* (1996, *Proc. Natl. Acad. Sci. USA*
10 93:7381-7386) reported the isolation of a monomer antagonistic to IL-1 with nanomolar affinity for the IL-1 receptor. This effort required construction and use of many phage displayed peptide libraries and sophisticated phage-panning procedures.

Wrighton *et al.* (1996, *Science* 273:458-464) and Livnah *et al.* (1996,
15 *Science* 273:464-471) reported dimer peptides that bind to the erythropoietin (EPO) receptor with full agonistic activity *in vivo*. These peptides are cyclical and have intra-peptide disulfide bonds; like the IL-1 receptor antagonist, they show no significant sequence identity to the natural ligand. Importantly, X-ray crystallography revealed that it was the spontaneous
20 formation of non-covalent peptide homodimer peptides that enabled the dimerization two EPO receptors.

WO 96/04557 reported the identification of peptides and antibodies that bound to active sites of biological targets, which were subsequently used in competition assays to identify small molecules that acted as agonist
25 or antagonists at the biological targets. Renschler *et al.* (1994, *Proc. Natl. Acad. Sci. USA* 91:3623-3627) reported synthetic peptide ligands of the antigen binding receptor that induced programmed cell death in human B-cell lymphoma.

Most recently, Cwirla *et al.* (1997, *Science* 276:1696-1698) reported
30 the identification of two families of peptides that bound to the human thrombopoietin (TPO) receptor and were competed by the binding of the

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natural TPO ligand. The peptide with the highest affinity, when dimerized by chemical means proved to be as potent an *in vivo* agonist as TPO, the natural ligand.

III. SUMMARY OF THE INVENTION

5 This invention relates to the identification of amino acid sequences that specifically recognize sites involved in IR or IGF-1R activation. Specific amino acid sequences are identified and their agonist or antagonist activity at IR and/or IGF-1R has been determined. Such sequences may be developed as potential therapeutics or as lead compounds to develop other
10 more efficacious ones. In addition, these sequences may be used in high-throughput screens to identify and provide information on small molecules that bind at these sites and mimic or antagonize the functions of insulin or IGF-1. Furthermore, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which can be used to identify
15 sequence variants that increase or otherwise modulate the binding and/or activity of the original peptide at IR or IGF-1R. The peptide sequences of the invention can also be combined to make dimer or other multimeric peptides, which can be used for screening, diagnostic, and thereapeutic applications as described herein.

20 In one aspect of this invention, large numbers of peptides have been screened for their IR and IGF-1R binding and activity characteristics. Analysis of their amino acid sequences has identified certain consensus sequences which may be used themselves or as core sequences in larger amino acid sequences conferring upon them agonist or antagonist activity.
25 Several generic amino acid sequences are disclosed which bind IR and/or IGF-1R with varying degrees of agonist or antagonist activity depending on the specific sequence of the various peptides identified within each motif group. Also provided are amino or carboxyl terminal extensions capable of modifying the affinity and/or pharmacological activity of the consensus
30 sequences when part of a larger amino acid sequence. Further provided

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are peptides containing more than one consensus sequence (e.g., dimer peptides).

The amino acid sequences of this invention which bind IR and/or IGF-1R include:

- 5 a. $X_1 X_2 X_3 X_4 X_5$ wherein X_1 , X_2 , X_4 and X_5 are aromatic amino acids, and X_3 is any polar amino acid (Formula 1; Group 1; A6 motif);
- b. $X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13}$ wherein X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} and X_{12} are any amino acid, and X_{10} and X_{13} are hydrophobic amino acids (Formula 2; Group 3; B6 motif);
- 10 c. $X_{14} X_{15} X_{16} X_{17} X_{18} X_{19} X_{20} X_{21}$ wherein X_{14} , and X_{17} are hydrophobic amino acids, X_{15} , X_{16} , X_{18} and X_{19} are any amino acid, and X_{20} and X_{21} are aromatic amino acids (Formula 3; reverse B6; revB6).
- d. $X_{22} X_{23} X_{24} X_{25} X_{26} X_{27} X_{28} X_{29} X_{30} X_{31} X_{32} X_{33} X_{34} X_{35} X_{36} X_{37} X_{38} X_{39} X_{40} X_{41}$ wherein X_{22} , X_{25} , X_{28} , X_{29} , X_{30} , X_{33} , X_{34} , X_{35} , X_{36} , X_{37} , X_{38} , X_{40} , and
15 X_{41} are any amino acid, X_{35} and X_{37} may be any amino acid for binding to IR, whereas X_{35} is preferably a hydrophobic amino acid and X_{37} is preferably glycine for binding to IGF-1R and possess agonist or antagonist activity. X_{23} and X_{26} are hydrophobic amino acids. This sequence further comprises at least two cysteine residues, preferably at X_{25} and X_{40} . X_{31} and X_{32} are small
20 amino acids (Formula 4; Group 7; E8 motif).
- e. $X_{42} X_{43} X_{44} X_{45} X_{46} X_{47} X_{48} X_{49} X_{50} X_{51} X_{52} X_{53} X_{54} X_{55} X_{56} X_{57} X_{58} X_{59} X_{60} X_{61}$ wherein X_{42} , X_{43} , X_{44} , X_{45} , X_{53} , X_{55} , X_{56} , X_{58} , X_{60} and X_{61} may be any amino acid, X_{43} , X_{46} , X_{49} , X_{50} , X_{54} are hydrophobic amino acids, X_{47} and X_{59} are preferably cysteines, X_{48} is a polar amino acid, and X_{51} , X_{52} and X_{57}
25 are small amino acids (Formula 5; mini F8 motif).
- f. $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$ wherein X_{62} , X_{65} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} , and X_{81} may be any amino acid; X_{63} , X_{70} , X_{74} are hydrophobic amino acids; X_{64} is a polar amino acid, X_{67} and X_{75} are aromatic amino acids and X_{72} and X_{79} are preferably cysteines capable of forming a loop (Formula 6; Group 2; D8 motif).
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g. H X₈₂ X₈₃ X₈₄ X₈₅ X₈₆ X₈₇ X₈₈ X₈₉ X₉₀ X₉₁ X₉₂ wherein X₈₂ is proline or alanine, X₈₃ is a small amino acid, X₈₄ is selected from leucine, serine or threonine, X₈₅ is a polar amino acid, X₈₆, X₈₈, X₈₉ and X₉₀ are any amino acid, and X₈₇, X₉₁ and X₉₂ are an aliphatic amino acid (Formula 7).

5 h. X₁₀₄ X₁₀₅ X₁₀₆ X₁₀₇ X₁₀₈ X₁₀₉ X₁₁₀ X₁₁₁ X₁₁₂ X₁₁₃ X₁₁₄ wherein at least one of the amino acids of X₁₀₆ through X₁₁₁, and preferably two, are tryptophan separated by three amino acids, and wherein at least one of X₁₀₄, X₁₀₅ and X₁₀₆ and at least one of X₁₁₂, X₁₁₃ and X₁₁₄ are cysteine (Formula 8); and

10 i. an amino acid sequence comprising the sequence JBA5: DYKDLCSWGVRIGWLAGLCPKK (SEQ ID NO:1541) or JBA5 minus FLAG® tag and terminal lysines: LCQSWGVRIGWLAGLCP (SEQ ID NO:1542) (Formula 9).

15 j. W X₁₂₃ G Y X₁₂₄ W X₁₂₅ X₁₂₆ (SEQ ID NO:1543) wherein X₁₂₃ is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X₁₂₄ is any amino acid, but preferably a charged or aromatic amino acid; X₁₂₅ is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X₁₂₆ is any amino acid, but preferably a small amino acid (Formula 10; Group 6 motif).

20 In one embodiment, peptides comprising a preferred amino acid sequence FYX₃WF (SEQ ID NO:1544) (Formula 1; Group 1; A6 motif) have been identified which competitively bind to sites on IR. Surprisingly, peptides comprising an amino acid sequence FYX₃WF (SEQ ID NO:1544) can possess agonist or antagonist activity at IR or IGF-1R.

25 This invention also identifies at least two distinct binding sites on IR and IGF-1R (Site 1 and Site 2) based on the differing ability of certain of the peptides to compete with one another and ligand for binding to IR or IGF-1R. Accordingly, this invention provides amino acid sequences that bind specifically to one or both sites of IR or IGF-1R. Furthermore, specific
30 amino acid sequences are provided which have agonist or antagonist

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characteristics based on their ability to bind to the specific sites of IR or IGF-1R.

5 In another embodiment of this invention, amino acid sequences which bind to one or more sites of IR or IGF-1R (e.g., Site 1 or Site 2) are covalently linked together to form multivalent ligands. These multivalent ligands are capable of forming complexes with a plurality of IR or IGF-1R. Either the same or different amino acid sequences are covalently bound together to form homo- or heterocomplexes.

10 In various aspects of the invention, monomer subunits are covalently linked at their N-termini or C-termini to form N-N, C-C, N-C, or C-N linked dimer peptides. In one example, dimer peptides are used to form receptor complexes bound through the same corresponding sites, e.g., Site 1-Site 1 or Site 2-Site 2 dimers. Alternatively, heterodimer peptides are used to bind to different sites on one receptor or to cause receptor complexing through
15 different sites, e.g., Site 1-Site 2 or Site 2-Site 1 dimers. In one novel aspect of the invention, Site 2-Site 1 dimers find use as insulin agonists, while certain Site 1-Site 2 dimers find use as insulin antagonists.

In various embodiments, insulin agonists comprise Site 1-Site 1 dimer peptide sequences S325, S332, S333, S335, S337, S353, S374-S376,
20 S378, S379, S381, S414, S415, and S418; whereas other insulin agonists comprise Site 2-Site 1 dimer peptide sequences S455, S457, S458, S467, S468, S471, S499, S510, S518, S519, and S520, as described herein below. In one preferred embodiment, an insulin agonist comprises the sequence of the S519 dimer peptide, which shows insulin-like activity in both
25 *in vitro* and *in vivo* assays.

The present invention also provides assays for identifying compounds that mimic the binding characteristics of insulin or IGF-1. Such compounds may act as antagonists or agonists of insulin or IGF-1 function in cell based assays.

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This invention further provides kits for identifying compounds that bind to IR and/or IGF-1R. Also provided are therapeutic compounds that bind the insulin receptor or the IGF-1 receptor.

5 Other embodiments of this invention are the nucleic acid sequences encoding the amino acid sequences of the invention. Also within the scope of this invention are vectors containing the nucleic acids and host cells which express the nucleic acids encoding the amino acid sequences which bind at IR and/or IGF-1R and possess agonist or antagonist activity.

10 This invention also provides amino acid sequences that bind to active sites of IR and/or IGF-1R and to identify structural criteria for conferring agonist or antagonist activity at IR or IGF-1R.

This invention further provides specific amino acid sequences that possess agonist, partial agonist, or antagonist activity at either IR or IGF-1R. Such amino acid sequences are potentially useful as therapeutics
15 themselves or may be used to identify other molecules, especially small organic molecules, which possess agonist or antagonist activity at IR or IGF-1R.

In addition, the present invention provides structural information derived from the amino acid sequences of this invention, which may be used
20 to construct other molecules possessing the desired activity at the relevant IR binding site.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1O; 2A-2E; 3A-3E; 4A-4I; 43A-43B, 44A-44B: Amino acid sequences identified by panning peptide libraries against IGF-1R and/or IR.
25 The amino acids are represented by their one-letter abbreviation. The ratios over background are determined by dividing the signal at 405 nm (E-Tag, IGF-1R, or IR) by the signal at 405 nm for non-fat milk. The IGF-1R/IR Ratio Comparison is determined by dividing the ratio of IGF-1R by the ratio of IR. The IR/IGF-1R Ratio Comparison is determined by dividing the ratio of IR by
30 the ratio of IGF-1R. HIT indicates binder; CAND indicates binder candidate;

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LDH indicates binding to lactate dehydrogenase (negative control); Sp/Irr indicates the ratio of specific binding over non-specific binding.

The design of each library is shown in the first line in bold. In the design, symbol 'X' indicates a random position, an underlined amino acid indicates a doped position at the nucleotide level, and other positions are held constant. Additional abbreviations in the B6H library are: 'O' indicates an NGY codon where Y is C or T; 'J' indicates an RHR codon where R is A or G, and H is A, C, or T; and 'U' indicates an VVY codon where V is A, C, or G, and Y is C or T. The 'h' in the 20E2 libraries indicates an NTN codon.

Symbols in the listed sequences include: Q indicates a position corresponding to a TAG stop codon; # indicates a position corresponding to a TAA stop codon; * indicates a position corresponding to a TGA stop codon; and ? indicates an unknown amino acid. It is believed that a W replaces the TGA stop codon when expressed. The Q residues represent translation read-through at TAG stop codons. Except for the 20C and A6L libraries, all libraries are designed with the short FLAG® epitope DYKD (SEQ ID NO:1545; Hopp *et al.*, 1988, *Bio/Technology* 6:1205-1210) at the N-terminus of the listed sequence and AAAGAP (SEQ ID NO:1546) at the C-terminus. The 20C and A6L libraries have the full length FLAG® epitope DYKDDDDK (SEQ ID NO:1547).

Figure 1A: Formula 1 motif peptide sequences obtained from a random 40mer library panned against IR (SEQ ID NOS:1-3).

Figure 1B: Formula 1 motif peptide sequence obtained from a random 40mer library panned against IGF-1R (SEQ ID NOS:4-6).

Figure 1C: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IR (SEQ ID NOS:7-29).

Figure 1D: Formula 1 motif peptide sequences obtained from a random 20mer library panned against IGF-1R (SEQ ID NOS:30-33).

Figure 1E: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain X₁₋₁₀NFYDWFVX₁₈₋₂₁ (SEQ ID NO:34; also referred to as "A6S") panned against IR (SEQ ID NOS:35-98).

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Figure 1F: Formula 1 motif peptide sequences obtained from a 21mer library constructed to contain $X_{1-10}NFYDWFVX_{18-21}$ (SEQ ID NO:34; also referred to as "A6S") panned against IGF-1R (SEQ ID NOS:99-166).

5 Figure 1G: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L" (SEQ ID NO:167)) panned against IR (SEQ ID NOS:168-216).

10 Figure 1H: Formula 1 motif peptide sequences obtained from a library constructed to contain variations outside the consensus core of the A6 peptide as indicated (referred to as "A6L" (SEQ ID NO:167)) panned against IGF-1R (SEQ ID NOS:217-244).

15 Figure 1I: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (SEQ ID NO:245) (as indicated) panned against IR (SEQ ID NOS:246-305).

Figure 1J: Formula 1 motif peptide sequences obtained from a library constructed to contain variations in the consensus core of the E4D peptide (SEQ ID NO:245) (as indicated) panned against IGF-1R (SEQ ID NOS:306-342).

20 Figure 1K: Formula 1 motif peptide sequences obtained from a library constructed using the sequence $X_{1-6}FHENFYDWFVRQVSX_{21-26}$ (SEQ ID NO:343; H2C-A) panned against IR (SEQ ID NOS:344-430).

25 Figure 1L: Formula 1 motif peptide sequences obtained from a library constructed using the sequence $X_{1-6}FHENFYDWFVRQVSX_{21-26}$ (SEQ ID NO:343; H2C-A) panned against IGF-1R (SEQ ID NOS:431-467).

Figure 1M: Formula 1 motif peptide sequences obtained from a library constructed using the sequence $X_{1-6}FHXXFYXWFX_{16-21}$ (SEQ ID NO:468; H2C-B) and panned against IR (SEQ ID NOS:469-575).

30 Figure 1N: Formula 1 motif peptide sequences obtained from a library constructed using the sequence $X_{1-6}FHXXFYXWFX_{16-21}$ (SEQ ID NO:468; H2C-B) and panned against IGF-1R (SEQ ID NOS:576-657).

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Figure 1O: Formula 1 motif peptide sequences obtained from other libraries panned against IR (SEQ ID NOS:658-712).

Figure 2A: Formula 4 motif peptide sequences identified from a random 20mer library panned against IR (SEQ ID NO:713).

5 Figure 2B: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO:713) as indicated (15% dope; referred to as "F815") panned against IR (SEQ ID NOS:714-796).

10 Figure 2C: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO:713) as indicated (15% dope; referred to as "F815") panned against IGF-1R (SEQ ID NOS:797-811).

15 Figure 2D: Formula 4 motif peptide sequences identified from a library constructed to contain variations in the F8 peptide (SEQ ID NO:713) as indicated (20% dope; referred to as "F820") panned against IR (SEQ ID NOS:812-861).

Figure 2E: Formula 4 motif peptide sequences identified from other libraries panned against IR (SEQ ID NOS:862-925).

20 Figure 3A: Formula 6 motif peptide sequences identified from a random 20mer library and panned against IR (SEQ ID NOS:926-928).

Figure 3B: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO:929) as indicated (15% dope; referred to as "D815") panned against IR (SEQ ID NOS:930-967).

25 Figure 3C: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO:929) as indicated (20% dope; referred to as "D820") panned against IR (SEQ ID NOS:968-1010).

30 Figure 3D: Formula 6 motif peptide sequences identified from a library constructed to contain variations in the D8 peptide (SEQ ID NO:929)

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as indicated (20% dope; referred to as "D820") panned against IGF-1R (SEQ ID NOS:1011-1059).

Figure 3E: Formula 6 motif peptide sequences identified from other libraries panned against IR (SEQ ID NOS:1060-1061).

5 Figure 4A: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IGF-1R (SEQ ID NOS:1062-1077).

Figure 4B: Formula 10 motif peptide sequences identified from random 20mer libraries panned against IR (SEQ ID NOS:1078-1082).

10 Figure 4C: Miscellaneous peptide sequences identified from a random 20mer library panned against IR (SEQ ID NOS:1083-1086).

Figure 4D: Miscellaneous peptide sequences identified from a random 40mer library panned against IR (SEQ ID NOS:1087-1088).

Figure 4E: Miscellaneous peptide sequences identified from a random 20mer library panned against IGF-1R (SEQ ID NOS:1089-1092).

15 Figure 4F: Miscellaneous peptide sequences identified from an X_{1-4} C X_{6-20} library and panned against IGF-1R (SEQ ID NOS:1093-1113).

Figure 4G: Miscellaneous peptide sequences identified from a library constructed to contain variations of the F8 peptide(SEQ ID NO:1114) as indicated (F815) panned against IGF-1R (SEQ ID NOS:1115-1118).

20 Figure 4H: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide(SEQ ID NO:1119) as indicated (referred to as "NNKH") panned against IR (SEQ ID NOS:1120-1142).

25 Figure 4I: Miscellaneous peptide sequences identified from a library constructed to contain variations in the F8A11 peptide(SEQ ID NO:1119) as indicated (referred to as "NNKH") panned against IGF-1R (SEQ ID NOS:1143-1154).

Figure 5A: Summary of specific representative amino acid sequences from Formulas 1, 4, 6, and 10 (SEQ ID NOS:1155-1180).

30 Figure 5B: Summary of specific representative amino acid sequences from Formulas 1, 4, 6, and 10 (SEQ ID NOS:1181-1220).

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Figure 6: Illustration of 2 binding site domains on IR based on competition data.

Figure 7: Schematic illustration of potential binding schemes to the multiple binding sites on IR.

5 Figure 8: Biopanning results and sequence alignments of Group 1 of IR-binding peptides (SEQ ID NOS:1221-1243). The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor. Absorbance signals are indicated by: +++++, >30X over background; +++, 15-30X; ++, 5-15X; +, 2-5X; and 0, <2X.

10 Figures 9A-9B: Biopanning results and sequence alignments of Groups 2, 6, and 7 of IR-binding peptides (SEQ ID NOS:1244-1261). The number of sequences found is indicated on the right side of the figure together with data on the phage binding to either IR or IGF-1R receptor. Absorbance signals are indicated by: +++++, >30X over background; +++, 15-30X; ++, 5-15X; +, 2-5X; and 0, <2X.

15 Figures 10A-10C: Insulin competition data determined for various monomer and dimer peptides. Figure 10A shows the competition curve. Figure 10B shows the symbol key for the peptides. Figure 10C shows the description of the peptides.

20 Figures 11A-11D: Insulin competition data determined for various monomer and dimer peptides. Figure 11A shows the competition curve. Figure 11B shows the symbol key for the peptides. Figure 11C shows the description of the peptides. Figure 11D shows IR binding affinity for the peptides.

25 Figures 12A-12D: Results of free fat cell assays for truncated synthetic RP9 monomer peptides, S390 and S394. Figure 12A shows the results for peptide S390. Figure 12B shows the results for peptide S394. Figure 12C shows the amino acid sequence of peptides S390 and S394 (SEQ ID NOS:1794 and 1788, respectively in order of appearance). Figure 12D shows the results for full-length RP9 peptide.

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Figures 13A-13C: Results of free fat cell assays for truncated synthetic RP9 dimer peptides, S415 and S417. Figure 13A shows the results for peptide S415. Figure 13B shows the results for peptide S417. Figure 13C shows the amino acid sequence of peptides S415 and S417 (SEQ ID NOS:1795-1796).

Figures 14A-14C: Results of free fat cell assays for RP9 homodimer peptides, 521 and 535. Figure 14A shows the results for peptide 521. Figure 14B shows the results for peptide 535. Figure 14C shows the amino acid sequence of peptides 521 and 535.

Figures 15A-15C: Results of free fat cell assays for RP9-D8 heterodimer peptides, 537 and 538. Figure 15A shows the results for peptide 537. Figure 15B shows the results for peptide 538. Figure 15C shows the amino acid sequence of peptides 537 and 538.

Figures 16A-16C: Results of free fat cell assays for RP9-D8 heterodimer peptides 537 and 538. Figure 16A shows the results for peptide 537. Figure 16B shows the results for peptide 538. Figure 16C shows the amino acid sequence of peptides 537 and 538.

Figures 17A-17B: Results of free fat cell assays for D8-RP9 heterodimer peptide, 539. Figure 17A shows the results for peptide 539. Figure 17B shows the amino acid sequence of peptide 539.

Figures 18A-18D: Results of free fat cell assays for Site 1/Site 2 dimer peptides with constituent monomer peptides with Site 1-Site 2 C-N (Figure 18A), Site 1-Site 2, N-N (Figure 18B), Site 1-Site 2, C-C (Figure 18C), and Site 2-Site 1, C-N (Figure 18D) orientations and linkages, respectively.

Figures 19A-19B: Results of human insulin receptor kinase assays for various monomer and dimer peptides. Figure 19A shows the substrate phosphorylation curve. Figure 19B shows the EC₅₀ values.

Figures 20A-20B: Results of human insulin receptor kinase assays for Site 1-Site 2 and Site 2-Site 1 dimer peptides. Figure 20A shows the substrate phosphorylation curve. Figure 20B shows the EC₅₀ values.

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Figures 21A-21B: Results of human insulin receptor kinase assays for Site 1-Site 2 and Site 2-Site 1 peptides. Figure 21A shows the substrate phosphorylation curve. Figure 21B shows the EC₅₀ values.

Figures 22A-22B: Results of time-resolved fluorescence resonance transfer assays for assessing the ability of various monomer and dimer peptides to compete with biotinylated RP9 monomer peptide for binding to soluble human insulin receptor-immunoglobulin heavy chain chimera. Figure 22A shows the binding curve. Figure 22B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figures 23A-23C: Results of time-resolved fluorescence resonance transfer assays indicating the ability of various monomer and dimer peptide to compete with biotinylated S175 monomer peptide or biotinylated RP9 monomer peptide for binding to soluble human insulin receptor-immunoglobulin heavy chain chimera. Figures 23A-23B show the binding curves. Figure 23C shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figures 24A-24B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptide to compete with fluorescein labeled RP9 monomer peptide for binding to soluble human insulin receptor ectodomain. Figure 24A shows the binding curve. Figure 24B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560 and 2001-2002, respectively in order of appearance).

Figures 25A-25B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluorescein labeled RP9 monomer peptide for binding to soluble human insulin mini-receptor. Figure 25A shows the binding curve. Figure 25B

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shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figures 26A-26B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluorescein labeled insulin for binding to soluble human insulin receptor ectodomain. Figure 26A shows the binding curve. Figure 26B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figures 27A-27B: Results of fluorescence polarization assays indicating the ability of various monomer and dimer peptides to compete with fluorescein labeled insulin for binding to soluble human insulin mini-receptor. Figure 27A shows the binding curve. Figure 27B shows the symbol key and description of the peptide sequences (SEQ ID NOS:2117, 1916-1917, 1558, 1994, 1960-1961, 2008, 1794, 2015-2016, 1560, and 2001-2002, respectively in order of appearance).

Figure 28: A schematic drawing for the construction of protein fusions of the maltose binding protein.

Figure 29: BIAcore analysis of competition binding between IR and maltose binding protein fusion peptides H2C-9aa-H2C, H2C, and H2C-3aa-H2C.

Figure 30: Stimulation of IR autophosphorylation in vivo by maltose binding protein fusion peptides.

Figures 31A-31C: Results of free fat cell assays for insulin and Site 2-Site 1 peptides, S519 and S520. Figure 31A shows the results for S519. Figure 31B shows the results for S520. Figure 31C shows the EC₅₀ values.

Figures 32A-32B: Results of human insulin receptor kinase assays for insulin and Site 2-Site 1 peptides S519 and S520. Figure 32A shows the substrate phosphorylation curve. Figure 32B shows the calculated Bestfit values.

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Figure 33: Results of *in vivo* experiments showing the effect of intravenous administration of Site 2-Site 1 peptide S519 in Wistar rats:

Figures 34A-34E: Results of phage competition studies with IGF-1 peptides RP9 (Site 1) and D815 (Site 2). Phage: RP9 (A6-like); RP6 (B6-like); D8B12 (Site 2); and D815 (Site 2); Peptides: RP9 and D815. Figures 34A-34B show the competition curves. Figures 34C-34E show the symbol keys and peptide groups.

Figure 35A-35E: Phage competition studies with Site 2-Site 1 dimer peptides containing 6- or 12-amino acid linkers. Phage: RP9, RP6, D8B12, and D815; Peptides: D815-6L-RP9 and D815-12L-RP9. Figures 35A-35B show the competition curves. Figures 35C-35E show the symbol keys and peptide groups.

Figure 36: Results of IGF-1 agonist assay using FDC-P2 cells. Site 1 peptides RP6, RP9, G33, and Site 2 peptide D815 were tested in the agonist assay.

Figure 37: Results of IGF-1 antagonist assay using FDCP-2 cells. Site 1 peptides RP6, RP9, G33, and Site 2 peptide D815 were tested in the antagonist assay.

Figure 38: Results of IGF-1 agonist assay using FDCP-2 cells. Site 1 peptides 20E2, S175, and RP9 were tested in the agonist assay.

Figures 39: Results of agonist and antagonist studies with peptide monomers and dimers. Monomers: D815 and RP9; Dimers: D815-6aa-RP9 and D815-12aa-RP9.

Figure 40: Results of agonist and antagonist studies with peptide monomers and dimers. Monomers: G33 and D815; Dimer: D815-6aa-G33.

Figure 41: Results of agonist and antagonist studies with peptide monomers and dimers. Monomers: G33, D815 and RP9; Dimers: D815-6aa-RP9 and D815-12aa-RP9.

Figure 42: IGF-1 standard curve using FDC-P2 cells.

Figures 43A-43B: Peptide monomers identified from G33 and RP6 secondary libraries panned against IGF-1R (SEQ ID NOS:1262-1432).

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Figure 43A shows peptides from G33 secondary library; Figure 43B shows peptides from RP6 secondary library.

Figures 44A-44B: Peptide dimers identified from libraries panned against IR or IGF-1R (SEQ ID NOS:1433-1540). Figure 44A shows dimer peptides panned against IR; Figure 44B shows dimer peptides panned against IGF-1R.

Figure 45: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide G33 (rG33) on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 46: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide D815 (rD815) on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 47: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide RP9 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 48: Results of heterogeneous time-resolved fluorometric assay showing the effect of recombinant peptide D815-6-G33 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 49: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide D815-6-RP9 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

Figure 50: Results of heterogeneous time-resolved fluorometric assays showing the effect of recombinant peptide D815-12-RP9 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

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Figure 51: Results of heterogeneous time-resolved fluorometric assays showing the effect of IGF-1 on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human IGF-1R (rhIGF-1R).

5 Figure 52: Results of time-resolved fluorescence resonance energy transfer assays showing the effect of Site 1 peptides, Site 2 peptides, and rhIGF-1 on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human IGF-1R.

10 Figure 53: Results of time-resolved fluorescence resonance energy transfer assays showing the effect of various peptide monomers and dimers on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human IGF-1R.

Figures 54A-54B, 55A-55B, 56A-56B, 57A-57B, 58A-58B, 59A-59B, 60A-60C, 61A-61B, 62A-62B, 63A-63B, and 64A-64B: Amino acid
 15 sequences identified by panning peptide libraries against IGF-1R. The amino acids are represented by their one-letter abbreviation. The ratios over background are determined by dividing the signal at 405 nm (E-Tag, IGF-1R, or IR) by the signal at 405 nm for non-fat milk. The IGF-1R/IR ratio comparison is determined by dividing the ratio of IGF-1R by the ratio of IR.
 20 The IR/IGF-1R ratio comparison is determined by dividing the ratio of IR by the ratio of IGF-1R. Sp/Irr = the ratio of specific binding over non-specific binding; LDH = lactate dehydrogenase (negative control).

Where included, the design of each library is shown in the first line in bold. In the design, symbol 'X' indicates a random position, an underlined
 25 amino acid indicates a doped position at the nucleotide level, and other positions are held constant. Symbols in the listed sequences include: Q indicates a position corresponding to a TAG stop codon; # indicates a position corresponding to a TAA stop codon; * indicates a position corresponding to a TGA stop codon; and ? indicates an unknown amino
 30 acid. The Q residues represent translation read-through at TAG stop codons. All libraries were designed with the short FLAG® Epitope DYKD

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(SEQ ID NO:1545; Hopp *et al.*, 1988, *Bio/Technology* 6:1205-1210) at the N-terminus of the listed sequence and an E-tag epitope (GAPVPYPDPLEPR; SEQ ID NO:XX) at the C-terminus.

5 Figures 54A-54B: Peptides identified from a RP6 secondary library panned against IGF-1R. The RP9 peptide is a Formula 1, Site 1 monomer.

Figures 55A-55B: Peptides identified from a RP9-NPB25 secondary library panned against IGF-1R. The RP9-NPB25 peptide is a Formula 2, Site 1 monomer with a 25 amino acid C-terminal extension.

10 Figures 56A-56B: Peptides identified from a RP30-IGF-NPB20 secondary library panned against IGF-1R. The RP30-IGF-NPB20 peptide is a Site 1, Formula 2 monomer with a 20 amino acid C-terminal extension.

Figures 57A-57B: Peptides identified from a NPB20-RP30-IGF secondary library panned against IGF-1R. The NPB20-RP30-IGF peptide is a Site 1, Formula 2 monomer with a 20 amino acid N-terminal extension.

15 Figures 58A-58B: Peptides identified from a D815 secondary library panned against IGF-1R. The D815 peptide is a Formula 6, Site 2 monomer.

Figures 59A-59B: Peptides identified from a RP6-D815 secondary library panned against IGF-1R. The RP6-D815 peptide is a Site 1-Site 2 dimer with no linker.

20 Figures 60A-60C: Peptides identified from a RP6-6aa-D815 secondary library panned against IGF-1R. The RP6-6aa-D815 peptide is a Site 1-Site 2 dimer with a 6 amino acid linker.

25 Figures 61A-61B: Peptides identified from a RP6-RP9 secondary library panned against IGF-1R. The RP6-RP9 peptide is a Site 1-Site 1 dimer with no linker.

Figures 62A-62B: Peptides identified from a RP6-6aa-RP9 secondary library panned against IGF-1R. The RP6-6aa-RP9 peptide is a Site 1-Site 1 dimer with a 6 amino acid linker.

30 Figures 63A-63B: Peptides identified from a D815-RP6 secondary library panned against IGF-1R. The D815-RP6 peptide is a Site 2-Site 1 dimer with no linker.

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Figures 64A-64B: Peptides identified from a D815-6aa-RP6 secondary library panned against IGF-1R. The D815-6aa-RP6 peptide is a Site 2-Site 1 dimer with a 6 amino acid linker.

Figures 65A-65F: Dose related increase in cell proliferation of MiaPaCa and MCF-7 cells as measured in response to IGF-1, IGF-2, and insulin. Cells were treated with either IGF-1, IGF-2, or insulin. Figure 65A: Results for MiaPaCa cells incubated with IGF-1; Figure 65B: MiaPaCa cells incubated with IGF-2; Figure 65C: MiaPaCa cells incubated with insulin; Figure 65D: MCF-7 cells incubated with IGF-1; Figure 65E: MCF-7 cells incubated with IGF-2; Figure 65F: MCF-7 cells incubated with insulin.

Figures 66A-66C: Peptide RP33-IGF competes with IGF-1 binding for binding to IGF-1R and antagonizes receptor activity in cell-based assays. For competition experiments, the ALPHAScreen assay format was used (see below). For antagonism assays, RP33-IGF was added to cells, cells were incubated with IGF-1, and cell number was determined. Figure 66A: Inhibition of IGF-1 binding as a function of RP33-IGF concentration. Figure 66B: Antagonism of IGF-1R in MCF-7 cells by peptide RP33-IGF. Figure 66C: Antagonism of IGF-1R in MiaPaCa cells by peptide RP33-IGF.

Figures 67A-67B: IGF-1 stimulates transient phosphorylation of IRS-1 in MCF-7 cells. Cells were stimulated with IGF-1 for 0, 2, 10, 30, 60 minutes and total protein was immunoprecipitated for each analysis. Figure 67A: Western blot analysis of endogenous IRS-1; Figure 67B: Western blot analysis of phosphorylated IRS-1; Lane 1: No addition; Lane 2: 2 minute time point; Lane 3: 10 minute time point; Lane 4: 30 minute time point; Lane 5: 60 minute time point.

Figures 68A-68B: Phosphorylation of IRS-1 in MCF-7 cells induced by IGF-1 is dose-dependant. Cells were exposed to increasing concentrations of IGF-1 and total protein was immunoprecipitated. Stimulation by 0.50 nM IGF-1 resulted in a sub-maximal level of phosphorylation that was consistently visualized in Western blot analysis. Figure 68A: Western blot analysis of endogenous IRS-1; Figure 68B:

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Western blot analysis of phosphorylated IRS-1; Lane 1: No addition; Lane 2: 0.05 nM IGF-1; Lane 3: 0.1 nM IGF-1; Lane 4: 5 nM IGF-1; Lane 5: 1 nM IGF-1; Lane 6: 0.5 nM IGF-1; Lane 7: 10 nM IGF-1; Lane 8: 50 nM IGF-1.

5 Figures 69A-69B: Blockade of IGF-1-induced phosphorylation of IRS-1 in MCF-7 cells by synthetic peptides RP6KK and RP33-IGF. Unrelated peptides KCB1 (VSIGECGGLRHHRVRELCLV; SEQ ID NO:XX) and DGI3-D1 (ECRWFRPWRCPLLSTGGGR; SEQ ID NO:XX) were used as negative controls. Figure 69A: Western blot analysis of expressed IRS-1; Figure 69B: Western blot analysis of phosphorylated IRS-1. Lane 1: no
10 addition; Lane 2: DGI3-D1; Lane 3: KCB1; Lane 4: IGF-1 plus DGI3-D1; Lane 5: IGF-1 plus KCB1; Lane 6: IGF-1 plus RP6KK; Lane 7: IGF-1 plus RP33-IGF; Lane 8: IGF-1.

 Figures 70A-70C: Peptides RP54 and RP52 compete with IGF-1 for
15 binding to IGF-1R, and act as antagonists in cell proliferation assays. For antagonism assays, RP54 or RP52 was added to cells, cells were incubated with IGF-1, and cell number was determined. Figure 70A: Antagonism of IGF-1R by RP54 in MCF-7 cells; Figure 70B: Antagonism of RP54 in MiaPaCa cells. Figure 70C: Antagonism of IGF-1 by RP52 in MCF-7 cells.

20 Figures 71A-71F: Peptide monomers with IGF-1R agonist or antagonist activity in MCF-7 or MiaPaCa cell proliferation assays compete against IGF-1 for binding to IGF-1R. Potencies of peptide competition were determined using the AlphaScreen assay format (see below). Figure 71A: RP60 peptide; Figure 71B: RP48 peptide; Figure 71C: sG33 peptide;
25 Figure 71D: C1 peptide; Figure 71E: L-RP9ex peptide; Figure 71F: 12-RP9ex peptide.

 Figures 72A-72E: Peptide dimers with IGF-1R agonist activity in MCF-7 or MiaPaCa cell proliferation assays compete with IGF-1 for binding to IGF-1R. Potencies of peptide competition were determined using the
30 AlphaScreen assay format (see below). Figure 72A: rRP30-IGF-12-D112 peptide (Site 1-Site 1); Figure 72B: rRP30-IGF-12-RP31-IGF peptide (Site

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1-Site 2); Figure 72C: rRP31-IGF-12-RP30-IGF peptide (Site 2-Site 1);
Figure 72D: rD112-12-RP30-IGF peptide (Site 1-Site 1); Figure 72E:
rD112-12-D112 peptide (Site 1-Site 1).

Figures 73A-73D: Peptide monomers with IGF-1R agonist activity in
5 MCF-7 or MiaPaCa cell proliferation assays. Figure 73A: RP60 peptide;
Figure 73B: RP48 peptide; Figure 73C: G33 peptide; Figure 73D: L-RP9ex
peptide.

Figures 74A-74I: Peptide dimers with IGF-1R agonist activity in
MCF-7 or MiaPaCa cell proliferation assays. Figure 74A: RP30-IGF-12-
10 D112 (Site 1-Site 1); Figure 74B: RP30-IGF-12-RP31-IGF (Site 1-Site 2);
Figure 74C: RP31-IGF-12-RP30-IGF (Site 2-Site 1); Figure 74D: D112-12-
RP30-IGF (Site 1-Site 1); Figure 74E: RP6-L-D8B12 (Site 1-Site 2); Figure
74F: D8B12-12-RP9 (Site 2-Site 1); Figure 74G: D112-12-D112 (Site 1-
Site 1); Figure 74H: RP9-12-RP9 (Site 1-Site 1); Figure 74I: RP9-L-RP6
15 (Site 1-Site 1).

V. DETAILED DESCRIPTION OF THE INVENTION

This invention relates to amino acid sequences comprising motifs that
bind to the insulin receptor (IR) and/or insulin-like growth factor receptor
(IGF-1R). In addition to binding to IR and/or IGF-1R, the amino acid
20 sequences also possess either agonist, partial agonist or antagonist activity
at IR or IGF-1R. In addition, the amino acid sequences bind to separate
binding sites (Sites 1 or 2) on IR or IGF-1R.

Although capable of binding to IR or IGF-1R at sites which participate
in conferring agonist or antagonist activity, the amino acid sequences are
25 not based on the native insulin or IGF-1 sequences, nor do they reflect an
obvious homology to any such sequences.

The amino acid sequences of the invention may be peptides,
polypeptides, or proteins. These terms as used herein should not be
considered limiting with respect to the size of the various amino acid
30 sequences referred to herein and which are encompassed within this

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invention. Thus, any amino acid sequence comprising at least one of the IR or IGF-1R binding motifs disclosed herein, and which binds to IR or IGF-1R is within the scope of this invention. In preferred embodiments, the amino acid sequences confer insulin or IGF-1 agonist or antagonist activity. The amino acid sequences of the invention are typically artificial, i.e., non-naturally occurring peptides, polypeptides, or fragments thereof. The amino acid sequences of the invention do not include insulin, insulin-like growth factors, antibodies against insulin receptors or insulin-like growth factor receptors, or fragments thereof. Amino acid sequences useful in the invention may be obtained through various means such as chemical synthesis, phage display, cleavage of proteins or polypeptides into fragments, or by any means which amino acid sequences of sufficient length to possess binding ability may be made or obtained.

The amino acid sequences provided by this invention should have an affinity for IR sufficient to provide adequate binding for the intended purpose. Thus, for use as a therapeutic, the peptide, polypeptide, or protein provided by this invention should have an affinity (K_d) of between about 10^{-7} to about 10^{-15} M. More preferably the affinity is 10^{-8} to about 10^{-12} M. Most preferably, the affinity is 10^{-10} to about 10^{-12} M. For use as a reagent in a competitive binding assay to identify other ligands, the amino acid sequence preferably has affinity for the receptor of between about 10^{-5} to about 10^{-12} M.

The present invention describes several different binding motifs, which bind to active sites on IR or IGF-1R. The binding motifs are defined based on the analysis of several different amino acid sequences and analyzing the frequency that particular amino acids or types of amino acids occur at a particular position of the amino acid sequence as described in the related applications of Beasley *et al.* International Application PCT/US00/08528, filed March 29, 2000, and Beasley *et al.*, U.S. Application Serial No. 09/538,038, filed March 29, 2000.

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Also included within the scope of this invention are amino acid sequences containing substitutions, additions, or deletions based on the teachings disclosed herein and which bind to IR or IGF-1R with the same or altered affinity. For example, sequence tags (e.g., FLAG® tags) or amino acids, such as one or more lysines, can be added to the peptide sequences of the invention (e.g., at the N-terminal or C-terminal ends) as described in detail herein. Sequence tags can be used for peptide purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy and amino terminal regions of the consensus motifs described below, which comprise sequence tags (e.g., FLAG® tags), or which contain amino acid residues that are not associated with a strong preference for a particular amino acid, may optionally be deleted providing for truncated sequences. Certain amino acids (e.g., C-terminal or N-terminal residues) such as lysine which promote the stability or biotinylation of the amino acids sequences may be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support.

Peptides that bind to IR or IGF-1R, and methods and kits for identifying such peptides, have been disclosed by Beasley *et al.*, International Application PCT/US00/08528 filed March 29, 2000 and Beasley *et al.*, U.S. Application Serial No. 09/538,038 filed March 29, 2000, which are incorporated by reference in their entirety.

A. Consensus Motifs

The following motifs have been identified as conferring binding activity to IR and/or IGF-1R:

1. $X_1X_2X_3X_4X_5$ (Formula 1; Group 1; the A6 motif) wherein X_1 , X_2 , X_4 and X_5 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X_1 and X_5 are phenylalanine and X_2 is tyrosine. X_3 may be

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any small polar amino acid, but is preferably selected from aspartic acid, glutamic acid, glycine, or serine, and is most preferably aspartic acid or glutamic acid. X_4 is most preferably tryptophan, tyrosine, or phenylalanine and most preferably tryptophan. Particularly preferred embodiments of the A6 motif are FYDWF (SEQ ID NO:1554) and FYEWF (SEQ ID NO:1555). The A6 motif possesses agonist activity at IGF-1R, but agonist or antagonist activity at IR depending on the identity of amino acids flanking A6. See Figure 5A.

Amino acid sequences that comprise the A6 motif and possess agonist activity at IR, include but are not limited to, D117/H2C: FHENFYDWFVRQVSKK (SEQ ID NO:1556); D117/H2 minus terminal lysines: FHENFYDWFVRQVS (SEQ ID NO:1557); RP9: GSLDESFYDWFERQLGKK (SEQ ID NO:1558); RP9 minus terminal lysines: GSLDESFYDWFERQLG (SEQ ID NO:1559); and S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560). Preferred RP9 sequences include GLADEDFYEWFERQLR (SEQ ID NO:1561), GLADELFYEWFDRQLS (SEQ ID NO:1562), GQLDEDFYEWFDRQLS (SEQ ID NO:1563), GQLDEDFYAWFDRQLS (SEQ ID NO:1564), GFMDESFYEWFERQLR (SEQ ID NO:1565), GFWDESFYAWFERQLR (SEQ ID NO:1566), GFMDESFYAWFERQLR (SEQ ID NO:1567), and GFWDESFYEWFERQLR (SEQ ID NO:1568). Non-limiting examples of Group 1 (Formula 1; A6) amino acid sequences are shown in Figures 1A-1O.

2. $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$ (Formula 2, Group 3; the B6 motif) wherein X_6 and X_7 are aromatic amino acids, preferably, phenylalanine or tyrosine. Most preferably, X_6 is phenylalanine and X_7 is tyrosine. X_8 , X_9 , X_{11} , and X_{12} may be any amino acid. X_{10} and X_{13} are hydrophobic amino acids, preferably leucine, isoleucine, phenylalanine, tryptophan or methionine, but more preferably leucine or isoleucine. X_{10} is most preferably isoleucine for binding to IR and leucine for binding to IGF-1R. X_{13} is most preferably leucine. Amino acid sequences of Formula 2 may function as an antagonist

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at the IGF-1R, or as an agonist at the IR. Preferred consensus sequences of the Formula 2 motif are $FYX_8X_9LX_{11}X_{12}L$ (SEQ ID NO:1569), $FYX_8X_9IX_{11}X_{12}L$ (SEQ ID NO:1570), $FYX_8AIX_{11}X_{12}L$ (SEQ ID NO:1571), and $FYX_8YFX_{11}X_{12}L$ (SEQ ID NO:1572).

5 Another Formula 2 motif for use with this invention comprises $FYX_8YFX_{11}X_{12}L$ (SEQ ID NO:1573) and is shown as Formula 2A ("NNRP") below:
 $X_{115}X_{116}X_{117}X_{118}FYX_8YFX_{11}X_{12}LX_{119}X_{120}X_{121}X_{122}$ (SEQ ID NO:1574) wherein $X_{115}-X_{118}$ and $X_{118}-X_{122}$ may be any amino acid which allows for binding to IR or IGF-1R. X_{115} is preferably selected from the group consisting of
10 tryptophan, glycine, aspartic acid, glutamic acid, and arginine. Aspartic acid, glutamic acid, glycine, and arginine are more preferred. Tryptophan is most preferred. The preference for tryptophan is based on its presence in clones at a frequency three to five fold higher than that expected over chance for a random substitution, whereas aspartic acid, glutamic acid and arginine are
15 present about two fold over the frequency expected for random substitution.

X_{116} preferably is an amino acid selected from the group consisting of aspartic acid, histidine, glycine, and asparagine. X_{117} and X_{118} are preferably glycine, aspartic acid, glutamic acid, asparagine, or alanine. More preferably X_{117} is glycine, aspartic acid, glutamic acid and asparagine
20 whereas X_{118} is more preferably glycine, aspartic acid, glutamic acid or alanine. X_8 when present in the Formula 2A motif is preferably arginine, glycine, glutamic acid, or serine. X_{11} when present in the Formula 2A motif is preferably glutamic acid, asparagine, glutamine, or tryptophan, but most preferably glutamic acid. X_{12} when present in the Formula 2A motif is
25 preferably aspartic acid, glutamic acid, glycine, lysine or glutamine, but most preferably aspartic acid. X_{119} is preferably glutamic acid, glycine, glutamine, aspartic acid or alanine, but most preferably glutamic acid. X_{120} is preferably glutamic acid, aspartic acid, glycine or glutamine, but most preferably glutamic acid. X_{121} is preferably tryptophan, tyrosine, glutamic acid,
30 phenylalanine, histidine, or aspartic acid, but most preferably tryptophan or tyrosine. X_{122} is preferably glutamic acid, aspartic acid or glycine; but most

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preferably glutamic acid. Preferred amino acid residue are identified based on their frequency in clones over two fold over that expected for a random event, whereas the more preferred sequences occur about 3-5 times as frequently as expected.

5 In certain cases, Formula 1 and Formula 2 amino acid sequences may also include two cysteine residues, which may be positioned either outside or inside the motif sequence (e.g., $X_1 X_2 X_3 X_4 X_5$ and $X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13}$), as described herein. The spacing between the cysteine residues preferably may vary from 3 amino acids, e.g., RP62
10 (CDFYCALSRLSGQPRDRMPNYPGTS; SEQ ID NO:XX) up to 19 amino acids, e.g., RP35 (DRDFCRFYERLTALVGGQVDGGWPC; SEQ ID NO:XX). Formula 1 and Formula 2 peptides may exhibit varying size and cysteine positioning. For example, Formula 2 peptide RP6 (TFYSCLASLLTGTPQPNRGPWERCR; SEQ ID NO:XX) and derivatives,
15 RP30-IGF, RP33-IGF, include two cysteine residues separated by 18 amino acids. In contrast, Formula 1 peptide G33 (GIISQSCPESFYDWFAGQVSDPWWCW; SEQ ID NO:XX) includes two cysteines separated by 17 amino acid residues. In certain Formula and Formula 2 peptides, the position and spacing of the cysteine residues was
20 found to be highly preferred in these peptides as determined by calculations of amino acid preferences from peptides obtained by biopanning of RP6 and G33 secondary libraries. Without wishing to be bound by theory, it is possible that the cysteine pairs observed in Formula 1 and Formula 2 amino acid sequences form cysteine loop structures.

25 3. $X_{14} X_{15} X_{16} X_{17} X_{18} X_{19} X_{20} X_{21}$ (Formula 3, reverse B6, revB6), wherein X_{14} and X_{17} are hydrophobic amino acids; X_{14} , X_{17} are preferably leucine, isoleucine, and valine, but most preferably leucine; X_{15} , X_{16} , X_{18} and X_{19} may be any amino acid; X_{20} is an aromatic amino acid, preferably tyrosine or histidine, but most preferably tyrosine; and X_{21} is an aromatic
30 amino acid, but preferably phenylalanine or tyrosine, and most preferably

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phenylalanine. For use as an IGF-1R binding ligand, an aromatic amino acid is strongly preferred at X₁₈.

4. X₂₂X₂₃X₂₄X₂₅X₂₆X₂₇X₂₈X₂₉X₃₀X₃₁X₃₂X₃₃X₃₄X₃₅X₃₆X₃₇X₃₈X₃₉X₄₀
 X₄₁ (Formula 4; Group 7; the F8 motif) wherein X₂₂, X₂₅, X₂₆, X₂₈, X₂₉, X₃₀,
 5 X₃₃, X₃₄, X₃₅, X₃₆, X₃₇, X₃₈, X₄₀, and X₄₁ are any amino acid. X₃₅ and X₃₇ may
 be any amino acid when the F8 motif is used as an IR binding ligand or as a
 component of an IR binding ligand, however for use as an IGF-1R binding
 ligand, glycine is strongly preferred at X₃₇ and a hydrophobic amino acid,
 particularly, leucine, is preferred at X₃₅. X₂₃ is a hydrophobic amino acid.
 10 Methionine, valine, leucine or isoleucine are preferred amino acids for X₂₃,
 however, leucine which is most preferred for preparation of an IGF-1R
 binding ligand is especially preferred for preparation of an IR binding ligand.
 At least one cysteine is located at X₂₄ through X₂₇, and one at X₃₉ or X₄₀.
 Together the cysteines are capable of forming a cysteine cross-link to create
 15 a looped amino acid sequence. In addition, although a spacing of 14 amino
 acids in between the two cysteine residues is preferred, other spacings may
 also be used provided binding to IGF-1R or IR is maintained. Accordingly,
 other amino acids may be substituted for the cysteines at positions X₂₄ and
 X₃₉ if the cysteines occupy other positions.
 20 In one embodiment, for example, the cysteine at position X₂₄ may
 occur at position X₂₇ which will produce a smaller loop provided that the
 cysteine is maintained at position X₃₉. These smaller looped peptides are
 described herein as Formula 5, infra. X₂₇ is any polar amino acid, but is
 preferably selected from glutamic acid, glutamine, aspartic acid, asparagine,
 25 or as discussed above cysteine. The presence of glutamic acid at position
 X₂₇ decreases binding to IR but has less of an effect on binding to IGF-1R.
 X₃₁ is any aromatic amino acid and X₃₂ is any small amino acid. For binding
 to IGF-1R, glycine or serine is preferred at position X₃₁, however, tryptophan
 is highly preferred for binding to IR. At position X₃₂, glycine is preferred for
 30 both IGF-1R and IR binding. X₃₆ is an aromatic amino acid. A preferred
 consensus sequence for F8 is X₂₂LCX₂₅X₂₆EX₂₈X₂₉X₃₀WGX₃₃X₃₄X₃₅

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X₃₆X₃₇X₃₈CX₄₀X₄₁ (SEQ ID NO:1575) whereas the amino acids are defined above. A more preferred F8 sequence is HLCVLEELFWGASLFGYCSG ("F8"; SEQ ID NO:1576). Amino acid sequences comprising the F8 sequence motif preferably bind to IR over IGF-1R. Figures 2A-2E list non-limiting examples of Formula 4 amino acid sequences.

5.

X₄₂X₄₃X₄₄X₄₅X₄₆X₄₇X₄₈X₄₉X₅₀X₅₁X₅₂X₅₃X₅₄X₅₅X₅₆X₅₇X₅₈X₅₉X₆₀X₆₁

(Formula 5; mini F8 motif) wherein X₄₂, X₄₃, X₄₄, X₄₅, X₅₃, X₅₅, X₅₆, X₅₈, X₆₀, and X₆₁ are any amino acid. X₄₃, X₄₆, X₄₉, X₅₀, and X₅₄ are hydrophobic amino acids, however, X₄₃ and X₄₆ are preferably leucine, whereas X₅₀ is preferably phenylalanine or tyrosine but most preferably phenylalanine. X₄₇ and X₅₉ are cysteines. X₄₈ is preferably a polar amino acid, i.e., aspartic acid or glutamic acid, but most preferably glutamic acid. Use of the small amino acid at position 54 may confer IGF-1R specificity. X₅₁, X₅₂, and X₅₇ are small amino acids, preferably glycine. A preferred consensus sequence for mini F8 is X₄₂X₄₃X₄₄X₄₅LCEx₄₉FGGX₅₃X₅₄X₅₅X₅₆G X₅₈CX₆₀X₆₁ (SEQ ID NO:1577). Amino acid sequences comprising the sequence of Formula 5 preferably bind to IGF-1R or IR.

6. X₆₂X₆₃X₆₄X₆₅X₆₆X₆₇X₆₈X₆₉X₇₀X₇₁X₇₂X₇₃X₇₄X₇₅X₇₆X₇₇X₇₈X₇₉X₈₀

X₈₁ (Formula 6; Group 2; the D8 motif) wherein X₆₂, X₆₅, X₆₈, X₆₉, X₇₁, X₇₃, X₇₆, X₇₇, X₇₈, X₈₀, and X₈₁ may be any amino acid. X₆₆ may also be any amino acid, however, there is a strong preference for glutamic acid. Substitution of X₆₆ with glutamine or valine may result in attenuation of binding. X₆₃, X₇₀, and X₇₄ are hydrophobic amino acids. X₆₃ is preferably leucine, isoleucine, methionine, or valine, but most preferably leucine. X₇₀ and X₇₄ are preferably valine, isoleucine, leucine, or methionine. X₇₄ is most preferably valine. X₆₄ is a polar amino acid, more preferably aspartic acid or glutamic acid, and most preferably glutamic acid. X₆₇ and X₇₅ are aromatic amino acids. Whereas tryptophan is highly preferred at X₆₇, X₇₅ is preferably tyrosine or tryptophan but most preferably tyrosine. X₇₂ and X₇₉ are

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cysteines that again are believed to form a loop which position amino acid may be altered by shifting the cysteines in the amino acid sequence.

D8 is most useful as an amino acid sequence having a preference for binding to IR as only a few D8 sequences capable of binding to IGF-1R over background have been detected. A preferred sequence for binding to IR is
 5 X₆₂LX₆₄X₆₅X₆₆WX₆₈X₆₉X₇₀X₇₁CX₇₃X₇₄X₇₅X₇₆X₇₇X₇₈CX₈₀X₈₁ (SEQ ID NO:1578). Examples of specific peptide sequences comprising this motif include D8: KWLDQEWAWVQCEVYGRGCP SKK (SEQ ID NO:1579); and D8 minus terminal lysines: KWLDQEWAWVQCEVYGRGCP S (SEQ ID
 10 NO:1580). Preferred D8 monomer sequences include SLEEWAQIQCEIYGRGCRY (SEQ ID NO:1581) and SLEEWAQIQCEIWGRGCRY (SEQ ID NO:1582). Preferred D8 dimer sequences include SLEEWAQIECEVYGRGCP S (SEQ ID NO:1583), and SLEEWAQIECEVWGRGCP S (SEQ ID NO:1584). Non-limiting examples
 15 of Group 2 (Formula 6; D8) amino acid sequences are shown in Figures 3A-3E.

7. HX₈₂X₈₃X₈₄X₈₅X₈₆X₈₇X₈₈X₈₉X₉₀X₉₁X₉₂ (Formula 7) wherein X₈₂ is proline or alanine but most preferably proline; X₈₃ is a small amino acid more preferably proline, serine or threonine and most preferably proline; X₈₄
 20 is selected from leucine, serine or threonine but most preferably leucine; X₈₅ is a polar amino acid preferably glutamic acid, serine, lysine or asparagine but more preferably serine; X₈₆ may be any amino acid but is preferably a polar amino acid such as histidine, glutamic acid, aspartic acid, or glutamine; X₈₇ is an aliphatic amino acid preferably leucine, methionine or
 25 isoleucine and most preferably leucine; amino acid X₈₈, X₈₉ and X₉₀ may be any amino acids; X₉₁ is an aliphatic amino acid with a strong preference for leucine as is X₉₂. Phenylalanine may also be used at position 92. A preferred consensus sequence of Formula 7 is HPPLSX₈₆LX₈₈X₈₉X₉₀LL (SEQ ID NO:1585). The Formula 7 motif binds to IR with little or no binding
 30 to IGF-1R.

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8. Another sequence is $X_{104}X_{105}X_{106}X_{107}X_{108}X_{109}X_{110}X_{111}X_{112}X_{113}X_{114}$ (Formula 8) which comprises eleven amino acids wherein at least one, and preferably two of the amino acids of X_{106} through X_{111} are tryptophan. In addition, it is also preferred that when two tryptophan amino acids are present in the sequence they are separated by three amino acids, which are preferably, in sequential order proline, threonine and tyrosine with proline being adjacent to the tryptophan at the amino terminal end. Accordingly, the most preferred sequence for $X_{107}X_{108}X_{109}X_{110}X_{111}$ is WPTYW (SEQ ID NO:1586). At least one of the three amino acids on the amino terminal (X_{104} , X_{105} , X_{106}) and at least one of the amino acids carboxy terminal (X_{112} , X_{113} , X_{114}) ends immediately flanking X_{107} - X_{111} are preferably a cysteine residue, most preferably at X_{105} and X_{113} respectively. Without being bound by theory, the cysteines are preferably spaced so as to allow for the formation of a loop structure. X_{104} and X_{114} are both small amino acids such as, for example, alanine and glycine. Most preferably, X_{104} is alanine and X_{114} is glycine. X_{105} may be any amino acid but is preferably valine. X_{112} is preferably asparagine. Thus, the most preferred sequence is ACVWPTYWNCG (SEQ ID NO:1587).

9. An amino acid sequence comprising JBA5: DYKDLCSWGVRIGWLAGLCPKK (SEQ ID NO:1541); or JBA5 without terminal lysines: LCQSWGVRIGWLAGLCP (SEQ ID NO:1542) (Formula 9). The Formula 9 motif is another motif believed to form a cysteine loop that possesses agonist activity at both IR and IGF-1R. Although IR binding is not detectable by ELISA, binding of Formula 9 to IR is competed by insulin and is agonistic.

10. $W X_{123} G Y X_{124} W X_{125} X_{126}$ (SEQ ID NO:1543) (Formula 10; Group 6) wherein X_{123} is selected from proline, glycine, serine, arginine, alanine or leucine, but more preferably proline; X_{124} is any amino acid, but preferably a charged or aromatic amino acid; X_{125} is a hydrophobic amino acid preferably leucine or phenylalanine, and most preferably leucine. X_{126} is any amino acid, but preferably a small amino acid. In one embodiment of

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the present invention, the Formula 10, Group 6 motif is WPGY (SEQ ID NO:1588). Examples of specific peptide sequences comprising this motif include E8: KVRGFQGGTWPGYEWLRNAKK (SEQ ID NO:1589); and E8 minus terminal lysines: KVRGFQGGTWPGYEWLRNAA (SEQ ID NO:1590). Preferred Group 6 sequences include WAGYEW (SEQ ID NO:1591), WEGYEW (SEQ ID NO:1592), WAGYEW (SEQ ID NO:1593), WEGYEW (SEQ ID NO:1594), and DSDWAGYEWFEQLD (SEQ ID NO:1595). Non-limiting examples of Group 6 amino acid sequences are shown in Figures 4A-4B.

The IR and IGF-1R binding activities of representative Group 1 (Formula 1; A6); Group 2 (Formula 6; D8); and Group 6 (Formula 10); and Group 7 (Formula 4; F8) amino acid sequences are summarized in Figures 8 and 9A-9B. Group 1 (Formula 1; A6) amino acid sequences contain the consensus sequence FyxWF (SEQ ID NO:1596), which is typically agonistic in cell-based assays. Group 2 (Formula 6; D8) amino acid sequences are composed of two internal sequences having a consensus sequence VYGR (SEQ ID NO:1597) and two cysteine residues each. Thus, Group 2 peptides are capable of forming a cyclic peptide bridged with a disulfide bond. Neither of these consensus sequences have any significant linear sequence similarities greater than 2 or 3 amino acids with mature insulin. Group 7 (Formula 4; F8) amino acid sequences are composed of two internal exemplary sequences which do not have any significant sequence homology, but have two cysteine residues 13-14 residues apart, thus being capable of forming a cyclic peptide with a long loop anchored by a disulfide bridge.

B. Amino And Carboxyl Terminal Extensions Modulate Activity of Motifs

In addition to the motifs stated above, the invention also provides preferred sequences at the amino terminal or carboxyl terminal ends which are capable of enhancing binding of the motifs to either IR, IGF-1R, or both.

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In addition, the use of the extensions described below does not preclude the possible use of the motifs with other substitutions, additions or deletions that allow for binding to IR, IGF-1R, or both.

1. Formula 1

5 Any amino acid sequence may be used for extensions of the amino terminal end of A6, although certain amino acids in amino terminal extensions may be identified which modulate activity. Preferred carboxy terminal extensions for A6 are A6-X₉₃X₉₄X₉₅X₉₆X₉₇ wherein X₉₃ may be any amino acid, but is preferably selected from the group consisting of alanine,
10 valine, aspartic acid, glutamic acid, and arginine, and X₉₄ and X₉₇ are any amino acid; X₉₅ is preferably glutamine, glutamic acid, alanine or lysine but most preferably glutamine. The presence of glutamic acid at X₉₅ however may confer some IR selectivity. Further, the failure to obtain sequences having an asparagine or aspartic acid at position X₉₅ may indicate that these
15 amino acids should be avoided to maintain or enhance sufficient binding to IR and IGF-1R. X₉₆ is preferably a hydrophobic or aliphatic amino acid, more preferably leucine, isoleucine, valine, or tryptophan but most preferably leucine. Hydrophobic residues, especially tryptophan at X₉₆ may be used to enhance IR selectivity.

20 2. Formula 2

B6 with amino terminal and carboxy terminal extensions may be represented as X₉₈X₉₉-B6-X₁₀₀. X₉₈ is optionally aspartic acid and X₉₉ is independently an amino acid selected from the group consisting of glycine, glutamine, and proline. The presence of an aspartic acid at X₉₈ and a
25 proline at X₉₉ is associated with an enhancement of binding for both IR and IGF-1R. A hydrophobic amino acid is preferred for the amino acid at X₁₀₀, an aliphatic amino acid is more preferred. Most preferably leucine, for IR and valine for IGF-1R. Negatively charged amino acids are preferred at both the amino and carboxy terminals of Formula 2A.

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3. Formula 3

An amino terminal extension of Formula 3 defined as $X_{101}X_{102}X_{103}$ -revB6 wherein X_{103} is a hydrophobic amino acid, preferably leucine, isoleucine or valine, and X_{102} and X_{101} are preferably polar amino acids, more preferably aspartic acid or glutamic acid may be useful for enhancing binding to IR and IGF-1R. No preference is apparent for the amino acids at the carboxy terminal end of Formula 3.

4. Formula 10

In one preferred embodiment, Formula 10 sequences $WX_{123}GYX_{124}WX_{125}X_{126}$ (SEQ ID NO:1543) can include an amino terminal extension comprising the sequence DSD and/or a carboxy terminal extension comprising the sequence EQLD (SEQ ID NO:1598).

C. IR Binding Preferences

As indicated above, the amino acid sequences containing the motifs of this invention may be constructed to have enhanced selectivity for either IR or IGF-1R by choosing appropriate amino acids at specific positions of the motifs or the regions flanking them. By providing amino acid preferences for IR or IGF-1R, this invention provides the means for constructing amino acid sequences with minimized activity at the non-cognate receptor. For example, the amino acid sequences disclosed herein with high affinity and activity for IR and low affinity and activity for IGF-1R are desirable as IR agonist as their propensity to promote undesirable cell proliferation, an activity of IGF-1 agonists, is reduced. Ratios of IR binding affinity to IGF-1R binding affinity for specific sequences are provided in Figures 1A-1O; 2A-2E; 3A-3E; 4A-4I; 44A-44B. As an insulin therapeutic, the IR/IGF-1R binding affinity ratio is preferably greater than 100. Conversely, for use as an IGF-1R therapeutic, the IR/IGF-1R ratio should be less than 0.01. Examples of peptides that selectively bind to IGF-1R are shown below.

TABLE 1

IGF-1R-SELECTIVE SEQUENCES

FORMULA 1 (Group 1; A6-like):

Ratios over Background

Comparisons

Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
A6L-0-E6-IR	1599	YRGLVLGRSSDGAGKVAFERPARIGQTVFAVNFYDFV	31.0	31.0	1.8	17.0	0.1
H2CA-4-G9-IGFR	1600	GIISQSCPEFYDFWAGQVSDPWGW	8.6	9.5	0.6	16.0	0.1
H2CA-4-H6-IGFR	1601	VGRASGFPEFYDFWGRQLSLQSGEQ	4.9	10.5	0.7	14.6	0.1
A6L-0-E4-IR	1602	YRGLVLGRISDGAG#VASEPPARIGRKVFAVNFYDFV	26.0	16.0	1.3	13.0	0.1
A6L-0-H3-IR	1603	YRGLVLGRISDGAGKAASERPARIGQKVAVNFYDFV	27.0	26.0	2.0	13.0	0.1
H2CA-4-F5-IGFR	1604	VGYQGGDENFYDFWIRQVSRGLGVQ	5.5	9.7	0.8	12.3	0.1
H2CA-4-H8-IGFR	1605	SACQDFCHENFYDFWAFARQVSGGAAYG	5.6	9.2	1.0	9.4	0.1
H2CA-4-F11-IGFR	1606	SAAQLFFQESFYDFWFLRQVASSQPN	3.5	6.8	1.0	6.7	0.1
H2CA-4-F6-IGFR	1607	AVRATRFDEAFYDFWFRQISDGQNK	3.9	7.3	1.1	6.4	0.2
H2CA-4-F10-IGFR	1608	VNQSGLHENFYDFWFERQVSHQGVNR	4.9	5.7	1.0	5.9	0.2
H2CA-1-A3-IGFR	1609	APDPSDFQEIFYDFWFRQVSRMPGGG	7.7	3.8	0.8	5.1	0.2
H2CA-3-C8-IGFR	1610	SSCDGAGHESFYDFWFRQVSGCRSV	15.1	5.6	1.2	4.8	0.2
H2CA-2-B9-IGFR	1611	RAGSSDFHEDFYDFWFRQVSLSLKKG	9.3	7.0	1.7	4.2	0.2
H2CA-4-H4-IGFR	1612	QAVQPGHEEFYDFWFRQVSTGVGGG	3.9	4.1	1.0	4.2	0.2
E4Dα-4-H2-IR	1613	GFREGNFYDFWFOAQT	37.8	33.9	8.2	4.1	0.2
H2CA-4-F7-IGFR	1614	SSIGGGFHENFYDFWFRQVSLQSPPLK	1.5	3.2	0.8	4.1	0.2
H2CA-3-D6-IGFR	1615	QSPVGSSEDFYDFWFRQVQAQGAHQ	8.3	9.0	2.2	4.0	0.3
H2CA-3-D8-IGFR	1616	NYRRQVFNGFYDFWFRQVFSLVTPG	10.9	7.2	1.8	4.0	0.3
H2CA-4-G11-IGFR	1617	TLDGGSFEEFYDFWFRQVSLYRTNPD	10.8	9.5	2.5	3.9	0.3
H2CA-4-F1-IGFR	1618	FYVQWGHENFYDFWFRQVSGSGAG	5.8	3.5	0.9	3.8	0.3
H2CA-3-D7-IGFR	1619	LRRQAPVEENFYDFWFRQVSGDRVGG	13.3	3.0	0.8	3.7	0.3
H2CA-1-A7-IGFR	1620	RCGRELYHSTFYDFWFRQVAGRTCPG	8.0	2.2	0.6	3.7	0.3
H2CA-2-B4-IGFR	1621	CCLLCRFQNFYDFWFCQGISRLRPL	3.5	4.1	1.1	3.6	0.3
H2CA-2-B3-IGFR	1622	PPLASDLLVQFYDFWFCQVSPPRGGG	7.7	3.8	1.0	3.6	0.3
H2CA-2-B2-IGFR	1623	GAPVDQLHEDFYDFWFRQVQAATG	4.1	3.4	1.0	3.5	0.3
E4Dα-2-D11-IR	1624	GFREGSFYDFWFOAQT	40.2	11.1	3.3	3.4	0.3
20E2Bβ-4-G6-IR	1625	SQAGSAFYAWFDQVLRITVHSA	22.4	6.2	1.9	3.3	0.3
H2CA-4-H9-IGFR	1626	RGAVAGFHDQFYDFWFRQVSRVHKFG	8.7	5.6	1.9	3.0	0.3

		Ratios over Background			Comparisons		
Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
H2CA-2-B11-IGFR	1627	ATCDAGFHEFYDNFALQVSDGCRQS	11.9	4.6	1.6	3.0	0.3
H2CA-3-E8-IGFR	1628	LGVEPFQNFYDNFVRQVSGAENAG	13.2	6.3	2.2	2.9	0.3
A6S-2-D11-IR	1629	EAASLSQDRNFYDFVQV	48.4	37.4	13.5	2.8	0.4
A6S-2-D1-IR	1630	VERSASSQDNFYDFVQVQIR	37.8	30.6	12.0	2.6	0.4
A6S-3-E2-IR	1631	TSEVQRSSQNFYDFVQAQA	33.1	24.7	9.8	2.5	0.4
H2CA-3-E11-IGFR	1632	HLADGFHEFYDNFVRQVSSNCDC	4.7	2.2	1.0	2.2	0.5
H2CA-3-C11-IGFR	1633	FRTLAQHDSFYDFDRQVSGAAGER	9.3	3.3	1.6	2.1	0.5
A6-PD1-IGFR	1634	SPHEDFYDNFDRQVSGSLKK					
H2C-PD1-IGFR(RP9)	1558	GSLDESFYDNFDRQVSGSLKK					

FORMULA 2 (Group 2; B6-like):

		Ratios over Background			Comparisons		
Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
20C-3-G3-IGFR	1635	TFYSCIASLLTGTQPNRGPWERC	33.1	32.3	1.2	27.0	<0.1
20C-4-C7-IGFR	1636	FFYDCLAALQGVARYHDLCAVEIT	35.3	28.0	1.3	21.8	<0.1
B6H α -1-B5-IR	1637	CCTTEMVMDARDPPFYHKLSELVTGG	41.5	20.5	1.0	20.5	0.0
R20 β -4-A6-IR	1638	RQSDAFYSGLWALIGLSDG	9.3	25.9	1.5	17.3	0.1
20E2B-1-A6-IGFR	1639	GVRAMSFYDALVSVLGLPSG	18.6	18.1	1.1	16.8	0.1
20 α -4-20A12-IR	1640	RLFYCGIQALGANLGYSGCV	48.6	39.9	2.4	16.6	0.1
20E2B β -4-G7-IR	1641	LQPCSGFYECIERLIGVKLSG	19.9	25.2	1.6	15.8	0.1
NRPY-4-B11-IR	1642	LKDGFDYFWQLHLS	4.1	18.7	1.2	15.5	0.1
20E2B-3-C6-IGFR	1643	VEGRGLFYDLRLQLLARRQNG	17.9	16.8	1.1	14.8	0.1
B6H α -1-A2-IR	1644	RGNDGGKGSDDPPFYHKLSELICGG	22.3	14.6	1.0	14.6	0.1
20E2A-4-F11-IGFR	1645	QGSASFYDAIDRLRMIRIG	21.3	18.8	1.3	14.6	0.1
B6H α -3-E9-IR	1646	RCEKQAEVGPSSDPFYHKMSLIGCR	44.6	24.2	1.7	14.2	0.1
20C-3-F6-IGFR	1647	DRDFCRFYERLTALVGGQVDGGWPC	33.5	26.1	1.9	14.1	0.1
20E2B-4-H3-IGFR	1648	KLHLMFYQGLQLVWAGL	11.2	14.8	1.1	13.9	0.1
20E2B-3-C2-IGFR	1649	GNGDGFYQLLSLLVGRDMHV	13.1	8.9	0.6	13.8	0.1
20C-3-A1-IGFR	1650	SSYCGDFYLMFLSLGLVASQLEEC	26.5	20.8	1.5	13.7	0.1
20E2B-3-E3-IGFR	1651	PDHKGFYAQLAQLIRGQLLS	22.4	16.3	1.3	13.1	0.1
R20 α -3-20E2-IR	1652	FYDAIDQLVRGSARAGTTRD	46.3	39.9	3.1	12.9	0.1
20E2B-4-H12-IGFR	1653	YSCGDGFYSLLSLLGLGGQFRC	6.5	9.7	0.8	12.8	0.1
B6H α -3-F11-IR	1654	RGMKKEVLVGSTDPFYHKLSELQGS	49.5	18.7	1.6	11.7	0.1

Ratios over Background Comparisons

Clone	SEQ ID NO.	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
20E2B-3-D2-IGFR	1655	IQEELTFYDLHLRLVRSELGS	20.7	12.4	1.1	11.7	0.1
20E2B-3-D8-IGFR	1656	GGTEVDFYRALERLVRGQIGL	20.4	17.7	1.6	11.3	0.1
20E2B-3-E8-IGFR	1657	LRIANLFYQRLWDLAFGGG	15.7	16.7	1.5	11.1	0.1
B6Hq-2-C4-IR	1658	RCGRW*AEMGAGDDPFYHKLSELVCG	20.7	9.9	0.9	11.0	0.1
R20q-4-20C11-IR	1659	DRAFYNGRLDLVGAVYGAWD	43.7	30.8	3.0	10.3	0.1
20E2B-4-F8-IGFR	1660	PVGQGFYEGLSRLVLGRGGW	12.3	7.3	0.8	9.7	0.1
20E2B-1-A11-IGFR	1661	RESTDGFYQYLLALVGGPVG	15.0	9.5	1.0	9.7	0.1
20E2B-3-D4-IGFR	1662	NSRDGGFYQLERLLGFPVTG	8.1	7.9	0.8	9.6	0.1
20E2B-2-B11-IGFR	1663	VVTPVNFYRALEALVRG.RLG	13.9	10.6	1.1	9.4	0.1
20E2B-3-C8-IGFR	1664	QPAPDGFYSALMKLIGRGVS	18.5	15.6	1.8	8.9	0.1
20E2B-2-B2-IGFR	1665	POTDLGFYQALRCVVIQAGCD	11.7	4.9	0.6	8.1	0.1
20E2B-4-F10-IGFR	1666	AQPCGGFYGLLEQLVGRSVC	19.0	17.3	2.2	7.8	0.1
20E2B-4-F9-IGFR	1667	QPDHSFYSLQELVGSERL	11.9	14.7	1.9	7.7	0.1
20C-3-A4-IGFR	1668	QYGCLLDLSLGVSFGWRRRCITA	17.7	8.8	1.2	7.6	0.1
20E2B-3-D11-IGFR	1669	LGVTDGFYAALGYLIHGVQF	14.3	12.2	1.6	7.6	0.1
20E2B-3-C11-IGFR	1670	CWM.DGFYAGLGLLTAGEGR	15.3	15.4	2.1	7.5	0.1
20E2B-2-B3-IGFR	1671	ICTGQGFYQVLCGLLRTSAR	9.1	5.3	0.7	7.4	0.1
20E2B-3-D12-IGFR	1672	QGNVLDYFGWIGRLIAKQSD	10.3	6.2	0.9	7.3	0.1
20E2B-3-E12-IGFR	1673	VATSQGFYSGLSSELLQGGNV	13.9	6.0	0.8	7.3	0.1
20E2B-2-B8-IGFR	1674	IWATGDFYRLLSQLVMGRVGT	17.4	5.7	0.8	7.2	0.1
NNRPY-4-A9-IR	1675	EGSGFYGYFFSLGLQG	3.0	10.0	1.4	7.1	0.1
20E2B-4-G11-IGFR	1676	RQGTGSFYLMLEQLLVGARGP	8.9	4.5	0.6	7.0	0.1
20E2B-3-D6-IGFR	1677	DSVGDNFYQLLESVLGGHVG	20.7	17.8	2.6	6.9	0.1
B6Hq-2-C7-IR	1678	RGIVAMVEATEVSGDHPFYHKLSELVQGS	45.1	6.7	1.0	6.7	0.1
20E2B-2-B7-IGFR	1679	LSSDGFYRALNLLQLQSAGR	18.0	6.1	0.9	6.7	0.1
20E2B-3-C4-IGFR	1680	ASSASGFYELLQRLAGLGV	23.4	20.4	3.3	6.2	0.2
20C-3-E4-IGFR	1681	FFYRCLSRLLGGQLGSLGSLCIGD	37.7	7.7	1.3	6.0	0.2
NNRPY-4-A1-IR	1682	IIGGFYSYFNSVLRIGT	9.7	10.9	1.8	6.0	0.2
20E2B-4-H8-IGFR	1683	PAGPCGFYCGLLHLHGQSP	7.2	5.3	0.9	5.9	0.2
20E2B-4-H9-IGFR	1684	RQGTGFYTCIQELIGFGDPD	4.5	5.2	0.9	5.6	0.2
B6Hq-2-C10-IR	1685	SGAKVIVTGDSDGDPFYHKLSELLQGS	46.9	5.8	1.1	5.3	0.2
20E2A-3-C7-IGFR	1686	VGTVAGFYDAIAQLVARASRV	17.6	5.4	1.1	5.1	0.2
20E2B-1-A8-IGFR	1687	TURSPTFYDWLEWVLTHTQGG	16.1	4.4	0.9	5.0	0.2
NNRPY-4-A7-IR	1688	RFPDPFYSYFVNLLGASA	2.5	6.3	1.3	4.9	0.2

Ratios over Background Comparisons

Clone	SEQ ID NO:	Sequence	E-Tag	IGF-1R	IR	IGF-1R/IR	IR/IGF-1R
B6H α -3-E8-IR	1689	RCKTAAVIVGRPADPFYHKLSELLQGG	47.6	5.3	1.1	4.8	0.2
B6H α -3-F10-IR	1690	GCVEWQKHWGASDPFYHKLSELLQGS	47.2	8.8	1.9	4.6	0.2
B6H α -2-D6-IR	1691	GRTMAVMAAGPDPFYHKLSELLQGG	33.5	4.4	1.0	4.4	0.2
B6H α -3-E7-IR	1692	GCAVVEAERSRGPDPFYHKLSELLQGC	47.0	5.6	1.3	4.3	0.2
B6H α -2-D1-IR	1693	GCEVIVEEGDSADPFYHKLSELLCQGS	11.7	5.4	1.3	4.2	0.2
20E2A-3-D10-IGFR	1694	MMVDFGYDALHQLVVAQSLG	20.6	6.9	1.8	3.9	0.3
20E2A-3-A12-IGFR	1695	LSVALSFYDALQQLVAGEGRW	16.1	4.3	1.1	3.9	0.3
B6H α -4-G8-IR	1696	GGTKAVAKVTRDDPFYHKLSELLQGS	32.3	6.1	1.7	3.6	0.3
B6L-4-D7-IR	1697	AETSVQVGWIRLQSVMPGEHWNTVDPFYHKLSELLRGSGA	14.3	4.8	1.4	3.4	0.3
B6H α -1-A3-IR	1698	SRKVAEMPDGDPFYHKLSELLASG	37.4	2.6	0.8	3.3	0.3
B6H α -3-F7-IR	1699	SRVAATKEKRPSPDPFYHKLSELLQGS	41.5	3.1	1.0	3.1	0.3
B6H α -2-D8-IR	1700	SSETAKMVTGTRDDPFYHKLSELLVQGS	19.3	3.0	1.0	3.0	0.3
B6H α -1-B3-IR	1701	GCITAEAGADPFYHKLSELLGSGS	33.1	3.2	1.1	2.9	0.3
B6H α -3-E5-IR	1702	RGDEEGWQENRRDDPFYHKLSELLFGGC	28.8	2.9	1.0	2.9	0.3
20E2A-4-G11-IGFR	1703	MMVFVSFYDAIDQLVCORIGC	20.7	3.3	1.3	2.6	0.4
20E2B β -3-C7-IR	1704	QSGSGDFYDWLSRLIRNGDGG	1.5	3.1	1.5	2.0	0.5
B6H α -3-E6-IR	1705	CGAKWTGTNDPFYHKLSELLQRG	18.2	2.3	1.2	1.9	0.5
20E2A-3-A3-IGFR	1706	GHYFGSFYDAIDQLVAGMLPG	5.2	3.0	1.5	1.9	0.5
B6L-4-A7-IR	1707	AGTPAQVG*NRLWSVMPGEHWNTVDPFYHKLSELLRESGA	11.6	3.4	1.9	1.8	0.6
B6H α -3-F1-IR	1708	CSMAVAEAGDDDDPFYHKLSELLCQGS	22.5	2.4	1.3	1.8	0.5
B6L-3-G6-IR	1709	VDTPAQVGNRLMSVGPGEHWYDDPFYH*LSELLRESGA	7.6	2.5	1.8	1.4	0.7
B6L-3-G5-IR	1710	AETSAQVGWQLWSVMPGDHWTSLDPPFYHKLSELLRESGA	11.5	2.0	1.4	1.4	0.7
20E2A-3-A4-IGFR	1711	AGSVTSFYDAMEQLVATGTS	16.8	2.5	1.8	1.4	0.7
B6-PD1-IGFR	1712	TDDGFYDALEQLVQGSKK					
20E2-PD1-IGFR (RP10)	1713	GSFYEALQRLVGEQGGKK					

FORMULA 10 (Group 6):

Clone	SEQ ID NO:	Sequence	Ratios over Background Comparisons			
			E-Tag	IGF-1R	IR	IGF-1R/IR
R20 β -4-E8-IR	1714	VRFGQGGTWPVGYEWRNAA	41.0	34.9	3.6	9.7
40F-4-D1-IGFR	1715	LSCLAYSRHGIWRPSTDLGLGRSVGEQSVSTRWGYDWFE	4.9	4.6	0.3	13.1
40F-4-B1-IGFR	1716	GLDHSDAVGVHLGFANPAQARGWEAGGLEDTWAGYDWL	4.1	3.0	0.2	13.1
40F-4-D10-IGFR	1717	W.GYAWLS	4.9	4.5	0.4	11.7

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Besides relative binding at IR or IGF-1R, relative efficacy at the cognate receptor is another important consideration for choosing a potential therapeutic. Thus, a sequence that is efficacious at IR but has little or no significant activity at IGF-1R may also be considered as an important IR therapeutic, irrespective of the relative binding affinities at IR and IGF-1R. For example, A6 selectivity for IR may be enhanced by including glutamic acid in a carboxyl terminal extension at position X₉₅. IR selectivity of the B6 motif may be enhanced by having a tryptophan or phenylalanine at X₁₁. Tryptophan at X₁₃ also favors selectivity of IR. A tryptophan amino acid at X₁₃ rather than leucine at that position also may be used to enhance selectivity for IR. In the reverse B6 motif, a large amino acid at X₁₅ favors IR selectivity. Conversely, small amino acids may confer specificity for IGF-1R. In the F8 motif, an L in position X₂₃ is essentially required for IR binding. In addition, tryptophan at X₃₁ is also highly preferred. At X₃₂, glycine is preferred for IR selectivity.

D. Multiple Binding Sites On IR And IGF-1R

The competition data disclosed herein reveals that at least two separate binding sites are present on IR and IGF-1R which recognize the different sequence motifs provided by this invention.

As shown in Figure 6, competition data indicate that peptides comprising the A6 motifs compete for binding to the same site on IR (Site 1) whereas the D8 motifs compete for a second site (Site 2). The identification of peptides that bind to separate binding sites on IR and IGF-1R provides for various schemes of binding to IR or IGF-1R to increase or decrease its activity. Examples of such schemes for IR are illustrated in Figure 7.

The table below shows sequences based on their groups, which bind to Site 1 or Site 2.

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TABLE 2

REPRESENTATIVE SITE 1 PEPTIDES

A6-like (FYxWF) (SEQ ID NO:1596):			
5	Clone	Sequence	SEQ ID NO:
	G3	KRGGGTFYEWFEALRKHGAGKK	1718
	H2	VTFTSAVFHENFYDWFVRQVSKK	1719
	H2C	PHENFYDWFVRQVSKK	1556
	A6S-IR3-E12	GRVDWLQRNANFYDWFVAELG	1560
10	A6S-IR4-G1	NGVERAGTGDNFYDWFVAQLH	1720
	H2CB-R3-B12	QSDSGTVHDRFYGWFRDTWAS	1721
	20E2A-R3-B11	GRFYGWFDQDAIDQLMPWGFDP	1722
	rB6-F6	RYGRWGLAQQFYDWFDR	1723
	E4Dα-1-B8-IR~	GFREGQRWYWFVAQVT	1724
15	H2CA-4-F11-IR	TYKARFLHENFYDWFNRQVSQYFGRV	1725
	H2CB-R3-D2	WTDVDGFHSGFYRWQONQWER	1726
	H2CB-R3-D12	VASGHVLHGQFYRWFDQFAL	1727
	H2CB-R4-H5	QARVGNVHQQFYEWFREVMQG	1728
	H2C-B-E8*	TGHRLLGLDEQFYWWFRDALSG	1729
20	H2CB-3-B6-IR~	VGDFCVSHDCFYGWFLRESMQ	1730
	A6S-IR2-C1	RMVFSTGAPQNFYDWFVQEW	1731
B6-like (FYxxLxxL) (SEQ ID NO:1732):			
	Clone	Sequence	
25	20C11	KDRAFYNGLRDLVGAVYGAWDKK	1733
	20E2	DYKDFYDAIDQLVRGSARAGGTRDKK	1734
	B62-R3-C7	EHWNTVDPFYFTLFEWLRESG	1735
	B62-R3-C10	EHWNTVDPFYQYFSELLRESG	1736
30	20E2B-3-B3-IR	AGVNAGFYRYFSTLLDWWDDQG	1737
	20E2-B-E3*	IQGWEPPFYGWFDVVAQMFE	1738
	20E2A-R4-F9	PPWGARFYDAIEQLVFDNLCC	1739
	RPNN-4-G6-HOLO*	RWPNFYGYFESLLTHFS	1740
	RPNN-4-F3-HOLO*	HYNAFYEYFQVLLAETW	1741
35	20E2A-R4-E2	IGRVRSFYDAIDKLFQSDWER	1742
	RPNN-2-C1-IR*	EGWDFYSYFSGLLASVT	1743
	20E2B-4-F12-IR	SVKEVQFYRYFYDLLQSEESG	1744
	20E2-B-E12	GNSGGSFYRYFQLLDSDGMS	1745
	20E2A-R3-B6	RDAGSSFYDAIDQLVCLTYFC	1746
40	Reverse B6-like (LxxLxxYF) (SEQ ID NO:1747):		
	Clone	Sequence	
	rB6-A12	LDALDRLMRYFEERPSL	1748
45	rB6-F9	PLAELWAYFEHSEQGRSSAH	1749
	rB6-4-E7-IR	LDPLDALLQYFWSVPGH	1750
	rB6-4-F9-IR	RGRLGSLSTQFYNWFAE	1751
	rB6-E6	ADELEWLLDYFMHQPRP	1752
	rB6-4-F12-IR	DGVLEELFSYFSATVGP	1753
50	Group 6 (WPxYxWL) (SEQ ID NO:1754):		
	Clone	Sequence	
	R20β-4-A4-IR	WPGYLFFEEALQDWRGSTED	1755
55	Peptides by design**:		
	Clone	Sequence	
	H2C-PD1-IR~	AAVHEQFYDWFADQYKK	1756
	A6S-PD1-IR~	QAPSNFYDWFVREWDKK	1757

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20E2-PD1-IR~	QSFYDYIEELLGGEWKK	1758
B6C-PD1-IR~	DPFYQGLWEWLRESGKK	1759

5 REPRESENTATIVE SITE 2 PEPTIDES (C-C LOOPS)

F8-derived (Long C-C loop):

	Clone	Sequence	SEQ ID NO:
	F8	HLCVLEELFWGASLFGYCSG	1760
10	F8-C12	FQSLLEELVWGAPLFRYGTG	1761
	F8-Des2	PLCVLEELFWGASLFGYCSG	1762
	F8-F12	PLCVLEELFWGASLFGQCSG	1763
	F8-B9	HLCVLEELFWGASLFGQCSG	1764
15	F8-B12	DLRVLCLELFGGAYVLGYCSE	1765
	NNKH-2B3	HRSVLKQLSWGASLFGQWAG	1766
	NNKH-2F9~	HLVGEELSWVALLGQWAR	1767
	NNKH-4H4~	APVSTEELRWGALLFGQWAG	1768

20

D8-derived (Small C-C loop):

	Clone	Sequence	SEQ ID NO:
	D8	KWLDQEWAWVQCEVYGRGCPSSK	1769
	D8-G1	QLEEEWAGVQCEVYGRECPSS	1770
25	D8-B5~	ALEEEWAWVQVRSIRSLPL	1771
	D8-A7	SLDQEWAWVQCEVYGRGCLS	1772
	D8-F1~	WLEHEWAQIQCELYGRGCTY	1773

Midi C-C loop:

	Clone	Sequence	
	D8-F10	GLEQGCPWVGLEVQCRGCPSS	1774
	F8-B12~	DLRVLCLELFGGAYVLGYCSE	1775
	F8-A9	PLWGLCLELFGGASLFGYCSSL	1776

35

**Based on analysis of entire panning data, amino acid preferences at each position were calculated to define these "idealized" peptides; * Peptides synthesized and currently being purified; ~ Peptides planned.

In various aspects of the present invention, amino acid sequences comprising Site 1 motifs may bind to Site 1 of IR or Site 1 of IGF-1R. Similarly, amino acids sequences comprising Site 2 motifs may bind to Site 2 of IR or Site 2 of IGF-1R. However, specific peptides may show higher binding affinity for IR than for IGF-1R, while other peptides may show higher binding affinity for IGF-1R than for IR. In addition, Site 1 and Site 2 on IR do not cross-talk, i.e., Site 1-binding sequences do not compete with Site 2-binding sequences at IR. In contrast, Site 1 and Site 2 on IGF-1R do show some cross-talk, suggesting an allosteric effect. These aspects are illustrated in the Examples described hereinbelow.

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E. Multivalent Ligands

This invention provides ligands that preferentially bind different sites on IR and IGF-1R. The A6 amino acid sequence motif confers binding to IR at Site 1 (Figure 6). The D8 amino acid sequence motif confers binding to IR at Site 2 (Figure 6). Accordingly, multimeric ligands may be prepared according to the invention by covalently linking amino acid sequences. Depending on the purpose intended for the multivalent ligand, amino acid sequences that bind the same or different sites may be combined to form a single molecule. Where the multivalent ligand is constructed to bind to the same corresponding site on different receptors, or different subunits of a receptor, the amino acid sequences of the ligand for binding to the receptors may be the same or different, provided that if different amino acid sequences are used, they both bind to the same site.

Multivalent ligands may be prepared by either expressing amino acid sequences which bind to the individual sites separately and then covalently linking them together, or by expressing the multivalent ligand as a single amino acid sequence which comprises within it the combination of specific amino acid sequences for binding.

Various combinations of amino acid sequences may be combined to produce multivalent ligands having specific desirable properties. Thus, agonists may be combined with agonists, antagonists combined with antagonists, and agonists combined with antagonists. Combining amino acid sequences that bind to the same site to form a multivalent ligand may be useful to produce molecules that are capable of cross-linking together multiple receptor units. Multivalent ligands may also be constructed to combine amino acid sequences which bind to different sites (Figure 7).

In view of the discovery disclosed herein of monomers having agonist properties at IR or IGF-1R, preparation of multivalent ligands may be useful to prepare ligands having more desirable pharmacokinetic properties due to the presence of multiple bind sites on a single molecule. In addition,

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combining amino acid sequences that bind to different sites with different affinities provides a means for modulating the overall potency and affinity of the ligand for IR or IGF-1R.

1. Construction of Hybrids

5 In one embodiment, hybrids of at least two peptides (e.g., dimer peptides) may be produced as recombinant fusion polypeptides, which are expressed in any suitable expression system. The polypeptides may bind the receptor as either fusion constructs containing amino acid sequences besides the ligand binding sequences or as cleaved proteins from which
10 signal sequences or other sequences unrelated to ligand binding are removed. Sequences for facilitating purification of the fusion protein may also be expressed as part of the construct. Such sequences optionally may be subsequently removed to produce the mature binding ligand. Recombinant expression also provides means for producing large quantities
15 of ligand. In addition, recombinant expression may be used to express different combinations of amino acid sequences and to vary the orientation of their combination, i.e., amino to carboxyl terminal orientation.

In one embodiment shown below (Figure 28), MBP-FLAG®-PEPTIDE-(GGS)_n (SEQ ID NO:1777)-PEPTIDE-E-TAG, a fusion construct
20 producing a peptide dimer comprises a maltose binding protein amino acid sequence (MBP) or similar sequence useful for enabling the affinity chromatography purification of the expressed peptide sequences. This purification facilitating sequence may then be attached to a FLAG® sequence to provide a cleavage site to remove the initial sequence. The
25 dimer then follows which includes the intervening linker and a tag sequence may be included at the carboxyl terminal portion to facilitate identification/purification of the expression of peptide. In the representative construct illustrated above, G and S are glycine and serine residues, which make up the linker sequence. As non-limiting examples, n can be 1, 2, 3, or
30 4 to yield a linker sequence of 3, 6, 9, and 12 amino acids, respectively.

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In addition to producing the dimer peptides by recombinant protein expression, dimer peptides may also be produced by peptide synthesis whereby a synthetic technique such as Merrifield synthesis (Merrifield, 1997), may be used to construct the entire peptide.

5 Other methods of constructing dimer peptides include introducing a linker molecule that activates the terminal end of a peptide so that it can covalently bind to a second peptide. Examples of such linkers include, but are not limited to, diaminopropionic acid activated with an oxyamino function. A preferred linker is a dialdehyde having the formula $O=CH-(CH_2)_n-CH=O$, wherein n is at least 2 to 6, but is preferably 6 to produce a
10 linker of about 25 to 30 angstroms in length. Other preferred linkers are shown in Table 3. Linkers may be used, for example, to couple monomers at either the carboxyl terminal or the amino terminal ends to form dimer peptides. Also, the chemistry can be inverted, i.e., the peptides to be
15 coupled can be equipped with aldehyde functions, either by oxidation with sodium periodate of an N-terminal serine, or by oxidation of any other vicinal hydroxy- or amino-groups, and the linker can comprise two oxyamino functions (e.g., at end of a polyethylene glycol linker) or amino groups which are coupled by reductive amination.

20 In specific embodiments, Site 1-Site 2 and Site 2-Site 1 orientations are possible. In addition, N-terminal to N-terminal (N-N); C-terminal to C-terminal (C-C); N-terminal to C-terminal (N-C); and C-terminal to N-terminal (C-N) linkages are possible. Accordingly, peptides may be oriented Site 1 to Site 2, or Site 2 to Site 1, and may be linked N-terminus to N-terminus, C-
25 terminus to C-terminus, N-terminus to C-terminus, or C-terminus to N-terminus. In certain cases, a specific orientation may be preferable to others, for example, for maximal agonist or antagonist activity.

In an unexpected and surprising result, the orientation and linkage of the monomer subunits has been found to dramatically alter dimer activity
30 (see Examples, below). In particular, certain Site 1/Site 2 heterodimer sequences show agonist or antagonist activity at IR, depending on

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orientation and linkage of the constituent monomer subunits. For example, a Site 1-Site 2 orientation (C-N linkage), e.g., the S453 heterodimer, shows antagonist activity at IR (Figure 18A; Table 7). In contrast, a Site 2-Site 1 orientation (C-N linkage), e.g., the S455 heterodimer, shows potent agonist activity at IR (Figure 18D; Table 7). Similarly, Site 1-Site 2 (C-N linkage) heterodimers, e.g., S425 and S459, show antagonist activity at IR (Table 7), while Site 1-Site 2 (C-C or N-N linkage) heterodimers, e.g., S432-S438, S454, and S456, show agonist activity (Table 7).

Whether produced by recombinant gene expression or by conventional chemical linkage technology, the various amino acid sequences may be coupled through linkers of various lengths. Where linked sequences are expressed recombinantly, and based on an average amino acid length of about 4 angstroms, the linkers for connecting the two amino acid sequences would typically range from about 3 to about 12 amino acids corresponding to from about 12 to about 48 Å. Accordingly, the preferred distance between binding sequences is from about 2 to about 50 Å. More preferred is 4 to about 40. The degree of flexibility of the linker between the amino acid sequences may be modulated by the choice of amino acids used to construct the linker. The combination of glycine and serine is useful for producing a flexible, relatively unrestrictive linker. A more rigid linker may be constructed by using amino acids with more complex side chains within the linkage sequence.

2. Characterization Of Specific Dimers

Specific dimers which are comprised of monomer subunits that both bind with high affinity to the same site on IR or IGF-1R (e.g., Site 1-Site 1 or Site 2-Site 2), or monomer subunits that bind to different sites on IR or IGF-1R (e.g., Site 1-Site 2 or Site 2-Site 1) are disclosed herein.

Other combinations of peptides are within the scope of this invention and may be determined as demonstrated in the examples described herein.

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F. Peptide Synthesis

Many conventional techniques in molecular biology, protein biochemistry, and immunology may be used to produce the amino acid sequences for use with this invention. The present invention encompasses the specific amino acid sequences shown in Figures 1-4, 8, and 9 and Table 7, *inter alia*, without additions (e.g., linker or spacer sequences) deletions, alterations, or modification. The present invention further encompasses variants that include additional sequences, altered sequences, and functional fragments thereof. In a preferred embodiment, the amino acid sequence variant or fragment shares at least one function characteristic (e.g., binding, agonist, or antagonist activity) of the reference sequence. Variant peptides include, for example, genetically engineered mutants, and may differ from the amino acid sequences shown in the figures and tables of the application by the addition, deletion, or substitution of one or more amino acid residues. Alterations may occur at the amino- or carboxy-terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. In addition, variants may comprise synthetic or non-naturally occurring amino acids in accordance with this invention.

Variant amino acid sequences can have conservative changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. More infrequently, a variant peptide can have non-conservative changes, e.g., substitution of a glycine with a tryptophan. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing binding or biological activity can be found using computer programs well-known in the art, for example, DNASTAR software (DNASTAR, Inc., Madison, WI). Guidance is also provided by the data disclosed herein. In particular, Figures 1-4, 8, 9, 43, 44, and Table 7, *inter alia*, teach which amino acid

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residues can be deleted, added, substituted, or modified, while maintaining the IR- or IGF-1R-related function(s) (e.g., binding, agonist, or antagonist activity) of the amino acid sequences.

For the purposes of this invention, the amino acids are grouped as follows: amino acids possessing alcohol groups are serine (S) and threonine (T). Aliphatic amino acids are isoleucine (I), leucine (L), valine (V), and methionine (M). Aromatic amino acids are phenylalanine (F), histidine (H), tryptophan (W), and tyrosine (Y). Hydrophobic amino acids are alanine (A), cysteine (C), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), leucine (L), methionine (M), arginine (R), threonine (T), valine (V), tryptophan (W), and tyrosine (Y). Negative amino acids are aspartic acid (D) and glutamic acid (E). The following amino acids are polar amino acids: cysteine (C), aspartic acid (D), glutamic acid (E), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), and threonine (T). Positive amino acids are histidine (H), lysine (K), and arginine (R). Small amino acids are alanine (A), cysteine (C), aspartic acid (D), glycine (G), asparagine (N), proline (P), serine (S), threonine (T), and valine (V). Very small amino acids are alanine (A), glycine (G) and serine (S). Amino acids likely to be involved in a turn formation are alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), glycine (G), histidine (H), lysine (K), asparagine (N), glutamine (Q), arginine (R), serine (S), proline (P), and threonine (T). As non-limiting examples, the amino acids within each of these defined groups may be substituted for each other in the formulas described above, as conservative substitutions, subject to the specific preferences stated herein.

Substantial changes in function can be made by selecting substitutions that are less conservative than those shown in the defined groups, above. For example, non-conservative substitutions can be made which more significantly affect the structure of the peptide in the area of the alteration, for example, the alpha-helical, or beta-sheet structure; the charge or hydrophobicity of the molecule at the target site; or the bulk of the side

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chain. The substitutions which generally are expected to produce the greatest changes in the peptide's properties are those where 1) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; 2) a cysteine or proline is substituted for (or by) any other residue; 3) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or 4) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) a residue that does not have a side chain, e.g., glycine.

10 Amino acid preferences have been identified for certain peptides and peptide groups of the present invention. For example, amino acid preferences for the RP9, D8, and Group 6 (Formula 10) peptides are shown in Tables 17-19, below. In some instances, cysteine pairs may also be preferred. For example, cysteine pairs are preferred in certain Formula 1 and Formula 2 sequences described herein. In accordance with the invention, the amino acid sequences of the invention may include two or more cysteine residues, which may be separated by at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 amino acids, and may be positioned inside or outside the Formula 1 or Formula 2 motif sequence. 20 Preferably, the cysteines are separated by 17 or 18 amino acids.

Variants also include amino acid sequences in which one or more residues are modified (i.e., by phosphorylation, sulfation, acylation, PEGylation, etc.), and mutants comprising one or more modified residues. Amino acid sequences may also be modified with a label capable of providing a detectable signal, either directly or indirectly, including, but not limited to, radioisotope, fluorescent, and enzyme labels. Fluorescent labels include, for example, Cy3, Cy5, Alexa, BODIPY, fluorescein (e.g., FluorX, DTAF, and FITC), rhodamine (e.g., TRITC), auramine, Texas Red, AMCA blue, and Lucifer Yellow. Preferred isotope labels include ³H, ¹⁴C, ³²P, ³⁵S, ³⁶Cl, ⁵¹Cr, ⁵⁷Co, ⁵⁸Co, ⁵⁹Fe, ⁹⁰Y, ¹²⁵I, ¹³¹I, and ¹⁸⁶Re. Preferred enzyme labels include peroxidase, β -glucuronidase, β -D-glucosidase, β -D-

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galactosidase, urease, glucose oxidase plus peroxidase, and alkaline phosphatase (see, e.g., U.S. Pat. Nos. 3,654,090; 3,850,752 and 4,016,043). Enzymes can be conjugated by reaction with bridging molecules such as carbodiimides, diisocyanates, glutaraldehyde, and the like. Enzyme labels can be detected visually, or measured by calorimetric, spectrophotometric, fluorospectrophotometric, amperometric, or gasometric techniques. Other labeling systems, such as avidin/biotin, Tyramide Signal Amplification (TSA™), are known in the art, and are commercially available (see, e.g., ABC kit, Vector Laboratories, Inc., Burlingame, CA; NEN® Life Science Products, Inc., Boston, MA).

1. Recombinant Synthesis of Peptides

To obtain recombinant peptides, DNA sequences encoding these peptides may be cloned into any suitable vectors for expression in intact host cells or in cell-free translation systems by methods well-known in the art (see Sambrook *et al.*, 1989). The particular choice of the vector, host, or translation system is not critical to the practice of the invention.

A large number of vectors, including bacterial, yeast, and mammalian vectors, have been described for replication and/or expression in various host cells or cell-free systems, and may be used for gene therapy as well as for simple cloning or protein expression. In one aspect of the present invention, an expression vector comprises a nucleic acid encoding a IR or IGF-1R agonist or antagonist peptide, as described herein, operably linked to at least one regulatory sequence. Regulatory sequences are known in the art and are selected to direct expression of the desired protein in an appropriate host cell. Accordingly, the term regulatory sequence includes promoters, enhancers and other expression control elements (see D.V. Goeddel (1990) *Methods Enzymol.* **185**:3-7). Enhancer and other expression control sequences are described in *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1983). It should be understood that the design of the expression vector

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may depend on such factors as the choice of the host cell to be transfected and/or the type of peptide desired to be expressed.

Several regulatory elements (e.g., promoters) have been isolated and shown to be effective in the transcription and translation of heterologous proteins in the various hosts. Such regulatory regions, methods of isolation, manner of manipulation, etc. are known in the art. Non-limiting examples of bacterial promoters include the β -lactamase (penicillinase) promoter; lactose promoter; tryptophan (trp) promoter; araBAD (arabinose) operon promoter; lambda-derived P_1 promoter and N gene ribosome binding site; and the hybrid tac promoter derived from sequences of the trp and lac UV5 promoters. Non-limiting examples of yeast promoters include the 3-phosphoglycerate kinase promoter, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter, galactokinase (GAL1) promoter, galactose epimerase promoter, and alcohol dehydrogenase (ADH1) promoter. Suitable promoters for mammalian cells include, without limitation, viral promoters, such as those from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Preferred replication and inheritance systems include M13, ColE1, SV40, baculovirus, lambda, adenovirus, CEN ARS, 2 μ m ARS and the like. While expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well-known in the art.

To obtain expression in eukaryotic cells, terminator sequences, polyadenylation sequences, and enhancer sequences that modulate gene expression may be required. Sequences that cause amplification of the gene may also be desirable. Furthermore, sequences that facilitate secretion of the recombinant product from cells, including, but not limited to, bacteria, yeast, and animal cells, such as secretory signal sequences and/or preprotein or proprotein sequences, may also be included. These sequences are well-described in the art. DNA sequences can be optimized, if desired, for more efficient expression in a given host organism or expression system. For example, codons can be altered to conform to the

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preferred codon usage in a given host cell or cell-free translation system using well-established techniques.

Codon usage data can be obtained from publicly-available sources, for example, the Codon Usage Database at <http://www.kazusa.or.jp/codon/>.
5 In addition, computer programs that translate amino acid sequence information into nucleotide sequence information in accordance with codon preferences (i.e., backtranslation programs) are widely available. See, for example, Backtranslate program from Genetics Computer Group (GCG), Accelrys, Inc., Madison, WI; and Backtranslation Applet from Entelechon
10 GmbH, Regensburg, Germany. Thus, using the peptide sequences disclosed herein, one of ordinary skill in the art can design nucleic acids to yield optimal expression levels in the translation system or host cell of choice.

Expression and cloning vectors will likely contain a selectable marker,
15 a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells that express the inserts. Typical selection genes encode proteins that 1) confer resistance to antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; 2) complement
20 auxotrophic deficiencies, or 3) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*. Markers may be an inducible or non-inducible gene and will generally allow for positive selection. Non-limiting examples of markers include the ampicillin resistance marker (i.e., beta-lactamase), tetracycline resistance
25 marker, neomycin/kanamycin resistance marker (i.e., neomycin phosphotransferase), dihydrofolate reductase, glutamine synthetase, and the like. The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts as understood by those of skill in the art.

30 Suitable expression vectors for use with the present invention include, but are not limited to, pUC, pBluescript (Stratagene), pET

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(Novagen, Inc., Madison, WI), and pREP (Invitrogen) plasmids. Vectors can contain one or more replication and inheritance systems for cloning or expression, one or more markers for selection in the host, e.g., antibiotic resistance, and one or more expression cassettes. The inserted coding
5 sequences can be synthesized by standard methods, isolated from natural sources, or prepared as hybrids. Ligation of the coding sequences to transcriptional regulatory elements (e.g., promoters, enhancers, and/or insulators) and/or to other amino acid encoding sequences can be carried out using established methods.

10 Suitable cell-free expression systems for use with the present invention include, without limitation, rabbit reticulocyte lysate, wheat germ extract, canine pancreatic microsomal membranes, *E. coli* S30 extract, and coupled transcription/translation systems (Promega Corp., Madison, WI). These systems allow the expression of recombinant peptides upon the
15 addition of cloning vectors, DNA fragments, or RNA sequences containing protein-coding regions and appropriate promoter elements.

Non-limiting examples of suitable host cells include bacteria, archaea, insect, fungi (e.g., yeast), plant, and animal cells (e.g., mammalian, especially human). Of particular interest are *Escherichia coli*, *Bacillus*
20 *subtilis*, *Saccharomyces cerevisiae*, SF9 cells, C129 cells, 293 cells, *Neurospora*, and immortalized mammalian myeloid and lymphoid cell lines. Techniques for the propagation of mammalian cells in culture are well-known (see, Jakoby and Pastan (Eds), 1979, *Cell Culture. Methods in Enzymology*, volume 58, Academic Press, Inc., Harcourt Brace Jovanovich,
25 NY). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, CHO cells, and WI38, BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other cell lines may be used, e.g., to provide higher expression, or other features.

Host cells can be transformed, transfected, or infected as appropriate
30 by any suitable method including electroporation, calcium chloride-, lithium chloride-, lithium acetate/polyethylene glycol-, calcium phosphate-, DEAE-

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dextran-, liposome-mediated DNA uptake, spheroplasting, injection, microinjection, microprojectile bombardment, phage infection, viral infection, or other established methods. Alternatively, vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the resulting RNA
5 introduced into the host cell by well-known methods, e.g., by injection (see, Kubo *et al.*, 1988, *FEBS Letts.* **241**:119). The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

Nucleic acids encoding the peptides of the invention may be isolated
10 directly from recombinant phage libraries (e.g., RAPIDLIB® or GRABLIB® libraries) described herein. Alternatively, the polymerase chain reaction (PCR) method can be used to produce nucleic acids of the invention, using the recombinant phage libraries as templates. Primers used for PCR can be
15 be designed to introduce appropriate new restriction sites, if desirable, to facilitate incorporation into a given vector for recombinant expression.

Nucleic acids encoding the peptides of the present invention can also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage *et al.*, 1981, *Tetra. Letts.* **22**:1859-1862, or the
20 triester method according to Matteucci *et al.*, 1981, *J. Am. Chem. Soc.*, **103**:3185, and can performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strands together under appropriate
25 conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

The nucleic acids encoding the peptides of the invention can be produced in large quantities by replication in a suitable host cell. Natural or synthetic nucleic acid fragments, comprising at least ten contiguous bases
30 coding for a desired amino acid sequence can be incorporated into recombinant nucleic acid constructs, usually DNA constructs, capable of

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introduction into and replication in a prokaryotic or eukaryotic cell. Usually the nucleic acid constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian or plant or other eukaryotic cells, cell lines, tissues, or organisms. The purification of nucleic acids produced by the methods of the present invention is described, for example, in Sambrook *et al.*, 1989; F.M. Ausubel *et al.*, 1992, *Current Protocols in Molecular Biology*, J. Wiley and Sons, New York, NY.

These nucleic acids can encode variant or truncated forms of the peptides as well as the reference peptides shown in Figures 1-4, 8, and 9 and Table 7, *inter alia*. Large quantities of the nucleic acids and peptides of the present invention may be prepared by expressing the nucleic acids or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used. Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. For example, insect cell systems (i.e., lepidopteran host cells and baculovirus expression vectors) are particularly suited for large-scale protein production.

Host cells carrying an expression vector (i.e., transformants or clones) are selected using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

For some purposes, it is preferable to produce the peptide in a recombinant system in which the peptide contains an additional sequence

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(e.g., epitope or protein) tag that facilitates purification. Non-limiting examples of epitope tags include c-myc, haemagglutinin (HA), polyhistidine (6X-HIS)(SEQ ID NO:1778), GLU-GLU, and DYKDDDDK (SEQ ID NO:1779) or DYKD (SEQ ID NO:1545; FLAG®) epitope tags. Non-limiting
5 examples of protein tags include glutathione-S-transferase (GST), green fluorescent protein (GFP), and maltose binding protein (MBP). In one approach, the coding sequence of a peptide can be cloned into a vector that creates a fusion with a sequence tag of interest. Suitable vectors include, without limitation, pRSET (Invitrogen Corp., San Diego, CA), pGEX
10 (Amersham Pharmacia Biotech, Inc., Piscataway, NJ), pEGFP (CLONTECH Laboratories, Inc., Palo Alto, CA), and pMAL™ (New England BioLabs, Inc., Beverly, MA) plasmids. Following expression, the epitope or protein tagged peptide can be purified from a crude lysate of the translation system or host cell by chromatography on an appropriate solid-phase matrix. In some
15 cases, it may be preferable to remove the epitope or protein tag (i.e., via protease cleavage) following purification.

Methods for directly purifying peptides from sources such as cellular or extracellular lysates are well-known in the art (see Harris and Angal, 1989). Such methods include, without limitation, sodium dodecylsulfate-
20 polyacrylamide gel electrophoresis (SDS-PAGE), preparative disc-gel electrophoresis, isoelectric focusing, high-performance liquid chromatography (HPLC), reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution, and combinations thereof. Peptides can be purified from many possible sources, for example,
25 plasma, body tissues, or body fluid lysates derived from human or animal, including mammalian, bird, fish, and insect sources.

Antibody-based methods may also be used to purify peptides. Antibodies that recognize these peptides or fragments derived therefrom can be produced and isolated. The peptide can then be purified from a
30 crude lysate by chromatography on an antibody-conjugated solid-phase matrix (see Harlow and Lane, 1998).

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2. Chemical Synthesis Of Peptides

Alternately, peptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or
5 classical solution synthesis. The peptides are preferably prepared by solid-phase peptide synthesis; for example, as described by Merrifield (1965; 1997).

According to methods known in the art, peptides can be chemically synthesized by commercially available automated procedures, including,
10 without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation, classical solution synthesis. In addition, recombinant and synthetic methods of peptide production can be combined to produce semi-synthetic peptides. The peptides of the invention are preferably prepared by solid phase peptide synthesis as described by
15 Merrifield, 1963, *J. Am. Chem. Soc.* **85**:2149; 1997. In one embodiment, synthesis is carried out with amino acids that are protected at the alpha-amino terminus. Trifunctional amino acids with labile side-chains are also protected with suitable groups to prevent undesired chemical reactions from occurring during the assembly of the peptides. The alpha-amino protecting
20 group is selectively removed to allow subsequent reaction to take place at the amino-terminus. The conditions for the removal of the alpha-amino protecting group do not remove the side-chain protecting groups.

The alpha-amino protecting groups are those known to be useful in the art of stepwise peptide synthesis. Included are acyl type protecting
25 groups, e.g., formyl, trifluoroacetyl, acetyl, aromatic urethane type protecting groups, e.g., benzyloxycarbonyl (Cbz), substituted benzyloxycarbonyl and 9-fluorenylmethyloxycarbonyl (Fmoc), aliphatic urethane protecting groups, e.g., t-butyloxycarbonyl (Boc), isopropylloxycarbonyl, cyclohexyloxycarbonyl, and alkyl type protecting groups, e.g., benzyl, triphenylmethyl. The
30 preferred protecting group is Boc. The side-chain protecting groups for Tyr

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include tetrahydropyranyl, tert-butyl, trityl, benzyl, Cbz, 4-Br-Cbz and 2,6-dichlorobenzyl. The preferred side-chain protecting group for Tyr is 2,6-dichlorobenzyl. The side-chain protecting groups for Asp include benzyl, 2,6-dichlorobenzyl, methyl, ethyl, and cyclohexyl. The preferred side-chain
5 protecting group for Asp is cyclohexyl. The side-chain protecting groups for Thr and Ser include acetyl, benzoyl, trityl, tetrahydropyranyl, benzyl, 2,6-dichlorobenzyl, and Cbz. The preferred protecting group for Thr and Ser is benzyl. The side-chain protecting groups for Arg include nitro, Tos, Cbz, adamantyloxycarbonyl, and Boc. The preferred protecting group for Arg is
10 Tos. The side-chain amino group of Lys can be protected with Cbz, 2-Cl-Cbz, Tos, or Boc. The 2-Cl-Cbz group is the preferred protecting group for Lys.

The side-chain protecting groups selected must remain intact during coupling and not be removed during the deprotection of the amino-terminus
15 protecting group or during coupling conditions. The side-chain protecting groups must also be removable upon the completion of synthesis, using reaction conditions that will not alter the finished peptide.

Solid phase synthesis is usually carried out from the carboxy-terminus by coupling the alpha-amino protected (side-chain protected)
20 amino acid to a suitable solid support. An ester linkage is formed when the attachment is made to a chloromethyl or hydroxymethyl resin, and the resulting peptide will have a free carboxyl group at the C-terminus. Alternatively, when a benzhydrylamine or p-methylbenzhydrylamine resin is used, an amide bond is formed and the resulting peptide will have a
25 carboxamide group at the C-terminus. These resins are commercially available, and their preparation has described by Stewart *et al.*, 1984, *Solid Phase Peptide Synthesis* (2nd Edition), Pierce Chemical Co., Rockford, IL.

The C-terminal amino acid, protected at the side chain if necessary and at the alpha-amino group, is coupled to the benzhydrylamine resin using
30 various activating agents including dicyclohexylcarbodiimide (DCC), N,N'-diisopropyl-carbodiimide and carbonyldiimidazole. Following the attachment

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to the resin support, the alpha-amino protecting group is removed using trifluoroacetic acid (TFA) or HCl in dioxane at a temperature between 0 and 25°C. Dimethylsulfide is added to the TFA after the introduction of methionine (Met) to suppress possible S-alkylation. After removal of the
5 alpha-amino protecting group, the remaining protected amino acids are coupled stepwise in the required order to obtain the desired sequence.

Various activating agents can be used for the coupling reactions including DCC, N,N'-diisopropyl-carbodiimide, benzotriazol-1-yl-oxy-tris-(dimethylamino) phosphonium hexa-fluorophosphate (BOP) and DCC-
10 hydroxybenzotriazole (HOBt). Each protected amino acid is used in excess (>2.0 equivalents), and the couplings are usually carried out in N-methylpyrrolidone (NMP) or in DMF, CH₂Cl₂ or mixtures thereof. The extent of completion of the coupling reaction is monitored at each stage, e.g., by the ninhydrin reaction as described by Kaiser *et al.*, 1970, *Anal. Biochem.*
15 **34**:595. In cases where incomplete coupling is found, the coupling reaction is repeated. The coupling reactions can be performed automatically with commercially available instruments.

After the entire assembly of the desired peptide, the peptide-resin is cleaved with a reagent such as liquid HF for 1-2 h at 0°C, which cleaves the
20 peptide from the resin and removes all side-chain protecting groups. A scavenger such as anisole is usually used with the liquid HF to prevent cations formed during the cleavage from alkylating the amino acid residues present in the peptide. The peptide-resin can be deprotected with TFA/dithioethane prior to cleavage if desired.

25 Side-chain to side-chain cyclization on the solid support requires the use of an orthogonal protection scheme which enables selective cleavage of the side-chain functions of acidic amino acids (e.g., Asp) and the basic amino acids (e.g., Lys). The 9-fluorenylmethyl (Fm) protecting group for the side-chain of Asp and the 9-fluorenylmethyloxycarbonyl (Fmoc) protecting
30 group for the side-chain of Lys can be used for this purpose. In these cases, the side-chain protecting groups of the Boc-protected peptide-resin

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are selectively removed with piperidine in DMF. Cyclization is achieved on the solid support using various activating agents including DCC, DCC/HOBt, or BOP. The HF reaction is carried out on the cyclized peptide-resin as described above.

5 3. Peptide Libraries

Peptide libraries produced and screened according to the present invention are useful in providing new ligands for IR and IGF-1R. Peptide libraries can be designed and panned according to methods described in detail herein, and methods generally available to those in the art (see, e.g.,
10 U.S. Patent No. 5,723,286 issued March 3, 1998 to Dower *et al.*). In one aspect, commercially available phage display libraries can be used (e.g., RAPIDLIB® or GRABLIB®, DGI BioTechnologies, Inc., Edison, NJ; Ph.D. C7C Disulfide Constrained Peptide Library, New England Biolabs). In another aspect, an oligonucleotide library can be prepared according to
15 methods known in the art, and inserted into an appropriate vector for peptide expression. For example, vectors encoding a bacteriophage structural protein, preferably an accessible phage protein, such as a bacteriophage coat protein, can be used. Although one skilled in the art will appreciate that a variety of bacteriophage may be employed in the present invention, in
20 preferred embodiments the vector is, or is derived from, a filamentous bacteriophage, such as, for example, f1, fd, Pf1, M13, etc. In particular, the fd-tet vector has been extensively described in the literature (see, e.g., Zacher *et al.*, 1980, *Gene* 9:127-140; Smith *et al.*, 1985, *Science* 228:1315-1317; Parmley and Smith, 1988, *Gene* 73:305-318).

25 The phage vector is chosen to contain or is constructed to contain a cloning site located in the 5' region of the gene encoding the bacteriophage structural protein, so that the peptide is accessible to receptors in an affinity enrichment procedure as described hereinbelow. The structural phage protein is preferably a coat protein. An example of an appropriate coat
30 protein is pIII. A suitable vector may allow oriented cloning of the

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oligonucleotide sequences that encode the peptide so that the peptide is expressed at or within a distance of about 100 amino acid residues of the N-terminus of the mature coat protein. The coat protein is typically expressed as a preprotein, having a leader sequence.

5 Thus, desirably the oligonucleotide library is inserted so that the N-terminus of the processed bacteriophage outer protein is the first residue of the peptide, i.e., between the 3'-terminus of the sequence encoding the leader protein and the 5'-terminus of the sequence encoding the mature protein or a portion of the 5' terminus. The library is constructed by cloning
10 an oligonucleotide which contains the variable region of library members (and any spacers, as discussed below) into the selected cloning site. Using known recombinant DNA techniques (see generally, Sambrook *et al.*, 1989, *Molecular Cloning, A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), an oligonucleotide may
15 be constructed which, *inter alia*; 1) removes unwanted restriction sites and adds desired ones; 2) reconstructs the correct portions of any sequences which have been removed (such as a correct signal peptidase site, for example); 3) inserts the spacer residues, if any; and/or 4) corrects the translation frame (if necessary) to produce active, infective phage.

20 The central portion of the oligonucleotide will generally contain one or more IR and/or IGF-1R binding sequences and, optionally, spacer sequences. The sequences are ultimately expressed as peptides (with or without spacers) fused to or in the N-terminus of the mature coat protein on the outer, accessible surface of the assembled bacteriophage particles. The
25 size of the library will vary according to the number of variable codons, and hence the size of the peptides, which are desired. Generally the library will be at least about 10^6 members, usually at least 10^7 , and typically 10^8 or more members. To generate the collection of oligonucleotides which forms a series of codons encoding a random collection of amino acids and which
30 is ultimately cloned into the vector, a codon motif is used, such as (NNK)_x, where N may be A, C, G, or T (nominally equimolar), K is G or T (nominally

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equimolar), and x is typically up to about 5, 6, 7, 8, or more, thereby producing libraries of penta-, hexa-, hepta-, and octa-peptides or larger. The third position may also be G or C, designated "S". Thus, NNK or NNS 1) code for all the amino acids; 2) code for only one stop codon; and 3) 5 reduce the range of codon bias from 6:1 to 3:1.

It should be understood that, with longer peptides, the size of the library that is generated may become a constraint in the cloning process. The expression of peptides from randomly generated mixtures of oligonucleotides in appropriate recombinant vectors is known in the art (see, 10 e.g., Oliphant *et al.*, Gene **44**:177-183). For example, the codon motif (NNK)₆ produces 32 codons, one for each of 12 amino acids, two for each of five amino acids, three for each of three amino acids and one (amber) stop codon. Although this motif produces a codon distribution as equitable as available with standard methods of oligonucleotide synthesis, it results in a 15 bias against peptides containing one-codon residues. In particular, a complete collection of hexacodons contains one sequence encoding each peptide made up of only one-codon amino acids, but contains 729 (3⁶) sequences encoding each peptide with only three-codon amino acids.

An alternative approach to minimize the bias against one-codon 20 residues involves the synthesis of 20 activated trinucleotides, each representing the codon for one of the 20 genetically encoded amino acids. These are synthesized by conventional means, removed from the support while maintaining the base and 5-OH-protecting groups, and activated by the addition of 3'O-phosphoramidite (and phosphate protection with b- 25 cyanoethyl groups) by the method used for the activation of mononucleosides (see, generally, McBride and Caruthers, 1983, *Tetrahedron Letters* **22**:245). Degenerate oligocodons are prepared using these trimers as building blocks. The trimers are mixed at the desired molar ratios and installed in the synthesizer. The ratios will usually be 30 approximately equimolar, but may be a controlled unequal ratio to obtain the over- to under-representation of certain amino acids coded for by the

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degenerate oligonucleotide collection. The condensation of the trimers to form the oligocodons is done essentially as described for conventional synthesis employing activated mononucleosides as building blocks (see, e.g., Atkinson and Smith, 1984, *Oligonucleotide Synthesis*, M.J. Gait, Ed., p. 35-82). This procedure generates a population of oligonucleotides for cloning that is capable of encoding an equal distribution (or a controlled unequal distribution) of the possible peptide sequences. Advantageously, this approach may be employed in generating longer peptide sequences, since the range of bias produced by the $(\text{NNK})_6$ motif increases by three-fold with each additional amino acid residue.

When the codon motif is $(\text{NNK})_x$, as defined above, and when x equals 8, there are 2.6×10^{10} possible octa-peptides. A library containing most of the octa-peptides may be difficult to produce. Thus, a sampling of the octa-peptides may be accomplished by constructing a subset library using up to about 10% of the possible sequences, which subset of recombinant bacteriophage particles is then screened. If desired, to extend the diversity of a subset library, the recovered phage subset may be subjected to mutagenesis and then subjected to subsequent rounds of screening. This mutagenesis step may be accomplished in two general ways: the variable region of the recovered phage may be mutagenized, or additional variable amino acids may be added to the regions adjoining the initial variable sequences.

To diversify around active peptides (i.e., binders) found in early rounds of panning, the positive phage can be sequenced to determine the identity of the active peptides. Oligonucleotides can then be synthesized based on these peptide sequences. The syntheses are done with a low level of all bases incorporated at each step to produce slight variations of the primary oligonucleotide sequences. This mixture of (slightly) degenerate oligonucleotides can then be cloned into the affinity phage by methods known to those in the art. This method produces systematic, controlled variations of the starting peptide sequences as part of a secondary library. It

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requires, however, that individual positive phage be sequenced before mutagenesis, and thus is useful for expanding the diversity of small numbers of recovered phage.

5 An alternate approach to diversify the selected phage allows the mutagenesis of a pool, or subset, of recovered phage. In accordance with this approach, phage recovered from panning are pooled and single stranded DNA is isolated. The DNA is mutagenized by treatment with, e.g., nitrous acid, formic acid, or hydrazine. These treatments produce a variety of damage to the DNA. The damaged DNA is then copied with reverse
10 transcriptase, which misincorporates bases when it encounters a site of damage. The segment containing the sequence encoding the receptor-binding peptide is then isolated by cutting with restriction nuclease(s) specific for sites flanking the peptide coding sequence. This mutagenized segment is then recloned into undamaged vector DNA, the DNA is
15 transformed into cells, and a secondary library according to known methods. General mutagenesis methods are known in the art (see Myers *et al.*, 1985, *Nucl. Acids Res.* **13**:3131-3145; Myers *et al.*, 1985, *Science* **229**:242-246; Myers, 1989, *Current Protocols in Molecular Biology Vol. I*, 8.3.1-8.3.6, F. Ausubel *et al.*, eds, J. Wiley and Sons, New York).

20 In another general approach, the addition of amino acids to a peptide or peptides found to be active, can be carried out using various methods. In one, the sequences of peptides selected in early panning are determined individually and new oligonucleotides, incorporating the determined sequence and an adjoining degenerate sequence, are synthesized. These
25 are then cloned to produce a secondary library. Alternatively, methods can be used to add a second IR or IGF-1R binding sequence to a pool of peptide-bearing phage. In accordance with one method, a restriction site is installed next to the first IR or IGF-1R binding sequence. Preferably, the enzyme should cut outside of its recognition sequence. The recognition site
30 may be placed several bases from the first binding sequence. To insert a second IR or IGF-1R binding sequence, the pool of phage DNA is digested

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and blunt-ended by filling in the overhang with Klenow fragment. Double-stranded, blunt-ended, degenerately synthesized oligonucleotides are then ligated into this site to produce a second binding sequence juxtaposed to the first binding sequence. This secondary library is then amplified and
5 screened as before.

While in some instances it may be appropriate to synthesize longer peptides to bind certain receptors, in other cases it may be desirable to provide peptides having two or more IR or IGF-1R binding sequences separated by spacer (e.g., linker) residues. For example, the binding
10 sequences may be separated by spacers that allow the regions of the peptides to be presented to the receptor in different ways. The distance between binding regions may be as little as 1 residue, or at least 2-20 residues, or up to at least 100 residues. Preferred spacers are 3, 6, 9, 12, 15, or 18 residues in length. For probing large binding sites or tandem
15 binding sites (e.g., Site 1 and Site 2 of IR), the binding regions may be separated by a spacer of residues of up to 20 to 30 amino acids. The number of spacer residues when present will typically be at least 2 residues, and often will be less than 20 residues.

The oligonucleotide library may have binding sequences which are
20 separated by spacers (e.g., linkers), and thus may be represented by the formula: $(NNK)_y - (abc)_n - (NNK)_z$ where N and K are as defined previously (note that S as defined previously may be substituted for K), and $y+z$ is equal to about 5, 6, 7, 8, or more, a, b and c represent the same or different nucleotides comprising a codon encoding spacer amino acids, n is up to
25 about 3, 6, 9, or 12 amino acids, or more. The spacer residues may be somewhat flexible, comprising oligo-glycine, or oligo-glycine-glycine-serine, for example, to provide the diversity domains of the library with the ability to interact with sites in a large binding site relatively unconstrained by attachment to the phage protein. Rigid spacers, such as, e.g., oligo-proline,
30 may also be inserted separately or in combination with other spacers, including glycine spacers. It may be desired to have the IR or IGF-1R

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binding sequences close to one another and use a spacer to orient the binding sequences with respect to each other, such as by employing a turn between the two sequences, as might be provided by a spacer of the sequence glycine-proline-glycine, for example. To add stability to such a turn, it may be desirable or necessary to add cysteine residues at either or both ends of each variable region. The cysteine residues would then form disulfide bridges to hold the variable regions together in a loop, and in this fashion may also serve to mimic a cyclic peptide. Of course, those skilled in the art will appreciate that various other types of covalent linkages for cyclization may also be used.

Spacer residues as described above may also be situated on either or both ends of the IR or IGF-1R binding sequences. For instance, a cyclic peptide may be designed without an intervening spacer, by having a cysteine residue on both ends of the peptide. As described above, flexible spacers, e.g., oligo-glycine, may facilitate interaction of the peptide with the selected receptors. Alternatively, rigid spacers may allow the peptide to be presented as if on the end of a rigid arm, where the number of residues, e.g., proline residues, determines not only the length of the arm but also the direction for the arm in which the peptide is oriented. Hydrophilic spacers, made up of charged and/or uncharged hydrophilic amino acids, (e.g., Thr, His, Asn, Gln, Arg, Glu, Asp, Met, Lys, etc.), or hydrophobic spacers of hydrophobic amino acids (e.g., Phe, Leu, Ile, Gly, Val, Ala, etc.) may be used to present the peptides to receptor binding sites with a variety of local environments.

Notably, some peptides, because of their size and/or sequence, may cause severe defects in the infectivity of their carrier phage. This causes a loss of phage from the population during reinfection and amplification following each cycle of panning. To minimize problems associated with defective infectivity, DNA prepared from the eluted phage can be transformed into appropriate host cells, such as, e.g., *E. coli*, preferably by electroporation (see, e.g., Dower *et al.*, *Nucl. Acids Res.* **16**:6127-6145), or

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well-known chemical means. The cells are cultivated for a period of time sufficient for marker expression, and selection is applied as typically done for DNA transformation. The colonies are amplified, and phage harvested for affinity enrichment in accordance with established methods. Phage
5 identified in the affinity enrichment may be re-amplified by infection into the host cells. The successful transformants are selected by growth in an appropriate antibiotic(s), e.g., tetracycline or ampicillin. This may be done on solid or in liquid growth medium.

For growth on solid medium, the cells are grown at a high density
10 (about 10^8 to 10^9 transformants per m^2) on a large surface of, for example, L-agar containing the selective antibiotic to form essentially a confluent lawn. The cells and extruded phage are scraped from the surface and phage are prepared for the first round of panning (see, e.g., Parmley and Smith, 1988, *Gene* 73:305-318). For growth in liquid culture, cells may be
15 grown in L-broth and antibiotic through about 10 or more doublings. The phage are harvested by standard procedures (see Sambrook *et al.*, 1989, *Molecular Cloning*, 2nd ed.). Growth in liquid culture may be more convenient because of the size of the libraries, while growth on solid media likely provides less chance of bias during the amplification process.

20 For affinity enrichment of desired clones, generally about 10^3 to 10^4 library equivalents (a library equivalent is one of each recombinant; 10^4 equivalents of a library of 10^9 members is $10^9 \times 10^4 = 10^{13}$ phage), but typically at least 10^2 library equivalents, up to about 10^5 to 10^6 , are incubated with a receptor (or portion thereof) to which the desired peptide is
25 sought. The receptor is in one of several forms appropriate for affinity enrichment schemes. In one example the receptor is immobilized on a surface or particle, and the library of phage bearing peptides is then panned on the immobilized receptor generally according to procedures known in the art. In an alternate scheme, a receptor is attached to a recognizable ligand
30 (which may be attached via a tether). A specific example of such a ligand is biotin. The receptor, so modified, is incubated with the library of phage and

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binding occurs with both reactants in solution. The resulting complexes are then bound to streptavidin (or avidin) through the biotin moiety. The streptavidin may be immobilized on a surface such as a plastic plate or on particles, in which case the complexes
5 (phage/peptide/receptor/biotin/streptavidin) are physically retained; or the streptavidin may be labeled, with a fluorophor, for example, to tag the active phage/peptide for detection and/or isolation by sorting procedures, e.g., on a fluorescence-activated cell sorter.

Phage that associate with IR or IGF-1R via non-specific interactions
10 are removed by washing. The degree and stringency of washing required will be determined for each receptor/peptide of interest. A certain degree of control can be exerted over the binding characteristics of the peptides recovered by adjusting the conditions of the binding incubation and the subsequent washing. The temperature, pH, ionic strength, divalent cation
15 concentration, and the volume and duration of the washing will select for peptides within particular ranges of affinity for the receptor. Selection based on slow dissociation rate, which is usually predictive of high affinity, is the most practical route. This may be done either by continued incubation in the presence of a saturating amount of free ligand, or by increasing the volume,
20 number, and length of the washes. In each case, the rebinding of dissociated peptide-phage is prevented, and with increasing time, peptide-phage of higher and higher affinity are recovered. Additional modifications of the binding and washing procedures may be applied to find peptides that bind receptors under special conditions. Once a peptide sequence that
25 imparts some affinity and specificity for the receptor molecule is known, the diversity around this binding motif may be embellished. For instance, variable peptide regions may be placed on one or both ends of the identified sequence. The known sequence may be identified from the literature, or may be derived from early rounds of panning in the context of the present
30 invention.

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G. Screening Assays

In another embodiment of this invention, screening assays to identify pharmacologically active ligands at IR and/or IGF-1R are provided. Ligands may encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Such ligands can comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. Ligands often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Ligands can also comprise biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs, or combinations thereof.

Ligands may include, for example, 1) peptides such as soluble peptides, including Ig-tailed fusion peptides and members of random peptide libraries (see, e.g., Lam *et al.*, 1991, *Nature* **354**:82-84; Houghten *et al.*, 1991, *Nature* **354**:84-86) and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids; 2) phosphopeptides (e.g., members of random and partially degenerate, directed phosphopeptide libraries, see, e.g., Songyang *et al.*, 1993, *Cell* **72**:767-778); 3) antibodies (e.g., polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, and single chain antibodies as well as Fab, F(ab')₂, Fab expression library fragments, and epitope-binding fragments of antibodies); and 4) small organic and inorganic molecules.

Ligands can be obtained from a wide variety of sources including libraries of synthetic or natural compounds. Synthetic compound libraries are commercially available from, for example, Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates

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(Merrimack, NH), and Microsource (New Milford, CT). A rare chemical library is available from Aldrich Chemical Company, Inc. (Milwaukee, WI). Natural compound libraries comprising bacterial, fungal, plant or animal extracts are available from, for example, Pan Laboratories (Bothell, WA). In addition, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides.

Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts can be readily produced. Methods for the synthesis of molecular libraries are readily available (see, e.g., DeWitt *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* **90**:6909; Erb *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* **91**:11422; Zuckermann *et al.*, 1994, *J. Med. Chem.* **37**:2678; Cho *et al.*, 1993, *Science* **261**:1303; Carell *et al.*, 1994, *Angew. Chem. Int. Ed. Engl.* **33**:2059; Carell *et al.*, 1994, *Angew. Chem. Int. Ed. Engl.* **33**:2061; and in Gallop *et al.*, 1994, *J. Med. Chem.* **37**:1233). In addition, natural or synthetic compound libraries and compounds can be readily modified through conventional chemical, physical and biochemical means (see, e.g., Blondelle *et al.*, 1996, *Trends in Biotech.* **14**:60), and may be used to produce combinatorial libraries. In another approach, previously identified pharmacological agents can be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, and the analogs can be screened for IR-modulating activity.

Numerous methods for producing combinatorial libraries are known in the art, including those involving biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide or peptide libraries, while the other four approaches are applicable to polypeptide, peptide, non-peptide oligomer, or small molecule libraries of compounds (K. S. Lam, 1997, *Anticancer Drug Des.* **12**:145).

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Libraries may be screened in solution by methods generally known in the art for determining whether ligands competitively bind at a common binding site. Such methods may including screening libraries in solution (e.g., Houghten, 1992, *Biotechniques* **13**:412-421), or on beads (Lam, 1991, *Nature* **354**:82-84), chips (Fodor, 1993, *Nature* **364**:555-556), bacteria or spores (Ladner U.S. Pat. No. 5,223,409), plasmids (Cull *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* **89**:1865-1869), or on phage (Scott and Smith, 1990, *Science* **249**:386-390; Devlin, 1990, *Science* **249**:404-406; Cwirla *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* **97**:6378-6382; Felici, 1991, *J. Mol. Biol.* **222**:301-310; Ladner, *supra*).

Where the screening assay is a binding assay, IR, or one of the IR-binding peptides disclosed herein, may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescent molecules, chemiluminescent molecules, enzymes, specific binding molecules, particles, e.g., magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin, etc. For the specific binding members, the complementary member would normally be labeled with a molecule that provides for detection, in accordance with known procedures.

A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, e.g., albumin, detergents, etc., which are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, antimicrobial agents, etc., may be used. The components are added in any order that produces the requisite binding. Incubations are performed at any temperature that facilitates optimal activity, typically between 4° and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Normally, between 0.1 and 1 h will be sufficient. In general, a plurality of assay mixtures is run in parallel with different test agent concentrations to obtain a differential

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response to these concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration or below the level of detection.

The screening assays provided in accordance with this invention are
5 based on those disclosed in International application WO 96/04557, which is incorporated herein in its entirety. Briefly, WO 96/04557 discloses the use of reporter peptides that bind to active sites on targets and possess agonist or antagonist activity at the target. These reporters are identified from recombinant libraries and are either peptides with random amino acid
10 sequences or variable antibody regions with at least one CDR region that has been randomized (rVab). The reporter peptides may be expressed in cell recombinant expression systems, such as for example in *E. coli*, or by phage display (see WO 96/04557 and Kay *et al.* 1996, *Mol. Divers.* 1(2):139-40, both of which are incorporated herein by reference). The
15 reporters identified from the libraries may then be used in accordance with this invention either as therapeutics themselves, or in competition binding assays to screen for other molecules, preferably small, active molecules, which possess similar properties to the reporters and may be developed as drug candidates to provide agonist or antagonist activity. Preferably, these
20 small organic molecules are orally active.

The basic format of an *in vitro* competitive receptor binding assay as the basis of a heterogeneous screen for small organic molecular replacements for insulin may be as follows: occupation of the active site of IR is quantified by time-resolved fluorometric detection (TRFD) with
25 streptavidin-labeled europium (saEu) complexed to biotinylated peptides (bP). In this assay, saEu forms a ternary complex with bP and IR (i.e., IR:bP:saEu complex). The TRFD assay format is well-established, sensitive, and quantitative (Tompkins *et al.*, 1993, *J. Immunol. Methods* 163:209-216). The assay can use a single-chain antibody or a biotinylated
30 peptide. Furthermore, both assay formats faithfully report the competition of the biotinylated ligands binding to the active site of IR by insulin.

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In these assays, soluble IR is coated on the surface of microtiter wells, blocked by a solution of 0.5% bovine serum albumin (BSA) and 2% non-fat milk in PBS, and then incubated with biotinylated peptide or rVab. Unbound bP is then washed away and saEu is added to complex with
5 receptor-bound bP. Upon addition of the acidic enhancement solution, the bound europium is released as free Eu^{3+} which rapidly forms a highly fluorescent and stable complex with components of the enhancement solution. The IR:bP bound saEu is then converted into its highly fluorescent state and detected by a detector such as Wallac Victor II (EG&G Wallac,
10 Inc.)

Phage display libraries can also be screened for ligands that bind to IR or IGF-1R, as described above. Details of the construction and analyses of these libraries, as well as the basic procedures for biopanning and selection of binders, have been published (see, e.g., WO 96/04557;
15 Mandecki *et al.*, 1997, *Display Technologies – Novel Targets and Strategies*, P. Guttry (ed), International Business Communications, Inc. Southborough, MA, pp. 231-254; Ravera *et al.*, 1998, *Oncogene* **16**:1993-1999; Scott and Smith, 1990, *Science* **249**:386-390); Grihalde *et al.*, 1995, *Gene* **166**:187-195; Chen *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* **93**:1997-
20 2001; Kay *et al.*, 1993, *Gene* **128**:59-65; Carcamo *et al.*, 1998, *Proc. Natl. Acad. Sci. USA* **95**:11146-11151; Hoogenboom, 1997, *Trends Biotechnol.* **15**:62-70; Rader and Barbas, 1997, *Curr. Opin. Biotechnol.* **8**:503-508; all of which are incorporated herein by reference).

The designing of mimetics to a known pharmaceutically active
25 compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesize or where it is unsuitable for a particular method of administration, e.g., peptides are generally unsuitable for active agents for oral compositions as they tend to be quickly degraded by
30 proteases in the alimentary canal. Mimetic design, synthesis, and testing

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are generally used to avoid large-scale screening of molecules for a target property.

There are several steps commonly taken in the design of a mimetic from a compound having a given target property. First, the particular parts
5 of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done by systematically varying the amino acid residues in the peptide (e.g., by substituting each residue in turn). These parts or residues constituting the active region of the compound are known as its "pharmacophore".

10 Once the pharmacophore has been found, its structure is modeled according to its physical properties (e.g., stereochemistry, bonding, size, and/or charge), using data from a range of sources (e.g., spectroscopic techniques, X-ray diffraction data, and NMR). Computational analysis, similarity mapping (which models the charge and/or volume of a
15 pharmacophore, rather than the bonding between atoms), and other techniques can be used in this modeling process.

In a variant of this approach, the three dimensional structure of the ligand and its binding partner are modeled. This can be especially useful where the ligand and/or binding partner change conformation on binding,
20 allowing the model to take account of this in the design of the mimetic.

A template molecule is then selected, and chemical groups that mimic the pharmacophore can be grafted onto the template. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesize, is likely to be
25 pharmacologically acceptable, does not degrade *in vivo*, and retains the biological activity of the lead compound. The mimetics found are then screened to ascertain the extent they exhibit the target property, or to what extent they inhibit it. Further optimization or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

30 This invention provides specific IR and IGF-1R amino acid sequences that function as either agonists or antagonists at IR and/or IGF-1R.

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Additional sequences may be obtained in accordance with the procedures described herein.

H. Use of the Peptides Provided by this Invention

The IR and IGF-1R agonist and antagonist peptides provided by this invention are useful as lead compounds for identifying other more potent or selective therapeutics, assay reagents for identifying other useful ligands by, for example, competition screening assays, as research tools for further analysis of IR and IGF-1R, and as potential therapeutics in pharmaceutical compositions. In one embodiment, one or more of the disclosed peptides can be provided as components in a kit for identifying other ligands (e.g., small, organic molecules) that bind to IR or IGF-1R. Such kits may also comprise IR or IGF-1R, or functional fragments thereof. The peptide and receptor components of the kit may be labeled (e.g., by radioisotopes, fluorescent molecules, chemiluminescent molecules, enzymes or other labels), or may be unlabeled and labeling reagents may be provided. The kits may also contain peripheral reagents such as buffers, stabilizers, etc. Instructions for use can also be provided.

In another embodiment, the peptide sequences provided by this invention can be used to design secondary peptide libraries, which are derived from the peptide sequences, and include members that bind to Site 1 and/or Site 2 of IR or IGF-1R. Such libraries can be used to identify sequence variants that increase or otherwise modulate the binding and/or activity of the original peptide at IR or IGF-1R, as described in the related applications of Beasley *et al.* International Application PCT/US00/08528, filed March 29, 2000, and Beasley *et al.*, U.S. Application Serial No. 09/538,038, filed March 29, 2000, in accordance with well-established techniques.

IR agonist amino acid sequences provided by this invention are useful as insulin analogs and may therefore be developed as treatments for diabetes or other diseases associated with a decreased response or

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production of insulin. For use as an insulin supplement or replacement, amino acid sequences include D117/H2C: FHENFYDWFVRQVSK (SEQ ID NO:1780); D117/H2C minus terminal lysine: FHENFYDWFVRQVS (SEQ ID NO:1557); D118: DYKDFYDAIQLVRSARAGGTRDKK (SEQ ID NO:1781);

5 D118 minus FLAG® tag and terminal lysines: FYDAIQLVRSARAGGTRD (SEQ ID NO:1782); D119: KDRAFYNGLRDLVGAVYGAWDKK (SEQ ID NO:1733); D119 minus terminal lysines: KDRAFYNGLRDLVGAVYGAWD (residues 1-21 of SEQ ID NO:1733); D116/JBA5: DYKDLCSWGVRIGWLAGLCPKK (SEQ ID NO:1541); D116/JBA5 minus

10 FLAG® tag and terminal lysines: LCQSWGVRIGWLAGLCP (SEQ ID NO:1542); D113/H2: DYKDVTFSTSAVFHENFYDWFVRQVSKK (SEQ ID NO:1783); D113/H2 minus FLAG® tag and terminal lysines: VTFTSAVFHENFYDWFVRQVS (SEQ ID NO:1784); and S175: GRVDWLQRNANFYDWFVAELG (SEQ ID NO:1560). Preferred peptide

15 dimer sequences are represented by S325, S332, S333, S335, S337, S353, S374-S376, S378, S379, S381, S414, S415, and S418 (see Table 7). Other preferred dimers sequences are represented by S455, S457, S458, S467, S468, S471, S499, S510, S518, S519, and S520 sequences (see Table 7). Especially preferred is the S519 dimer sequence, which shows *in vitro* and

20 *in vivo* activity comparable to insulin (see Figures 31A-C, 32A-B, and 33).

IGF-1R antagonist amino acid sequences provided by this invention are useful as treatments for cancers, including, but not limited to, breast, prostate, colorectal, and ovarian cancers. Human and breast cancers are responsible for over 40,000 deaths per year, as present treatments such as

25 surgery, chemotherapy, radiation therapy, and immunotherapy show limited success. The IGF-1R antagonist amino acid sequences disclosed herein are also useful for the treatment or prevention of diabetic retinopathy. Recent reports have shown that a previously identified IGF-1R antagonist can suppress retinal neovascularization, which causes diabetic retinopathy

30 (Smith *et al.*, 1999, *Nat. Med.* 5:1390-1395). Preferred IGF-1R antagonist

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amino acid sequences include those comprising the sequences of RP33-IGF and RP33K-IGF (Tables 24-26).

IGF-1R agonist amino acid sequences provided by this invention are useful for development as treatments for neurological disorders, including stroke and diabetic neuropathy. Reports of several different groups implicate IGF-1R in the reduction of global brain ischemia, and support the use of IGF-1 for the treatment of diabetic neuropathy (reviewed in Auer *et al.*, 1998, *Neurology* **51**:S39-S43; Apfel, 1999, *Am. J. Med.* **107**:34S-42S). The IGF-1R agonist peptides of the invention may be useful for enhancing the survival of cells and/or blocking apoptosis in cells. Preferred IGF-1R agonist amino acid sequences include those comprising the sequences of G33, RP48, RP60, and RP30-IGF-12-RP31-IGF (Tables 27-29).

I. Methods of Administration

The amino acid sequences of this invention may be administered as pharmaceutical compositions comprising standard carriers known in the art for delivering proteins and peptides and by gene therapy. Preferably, a pharmaceutical composition includes, in admixture, a pharmaceutically (i.e., physiologically) acceptable carrier, excipient, or diluent, and one or more of an IR or IGF-1R agonist or antagonist peptide, as an active ingredient. The preparation of pharmaceutical compositions that contain peptides as active ingredients is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions, however, solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified. The active therapeutic ingredient is often mixed with excipients that are pharmaceutically (i.e., physiologically) acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary

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substances such as wetting or emulsifying agents, pH-buffering agents, which enhance the effectiveness of the active ingredient.

An IR or IGF-1R agonist or antagonist peptide can be formulated into a pharmaceutical composition as neutralized physiologically acceptable salt
5 forms. Suitable salts include the acid addition salts (i.e., formed with the free amino groups of the peptide molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic
10 bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The pharmaceutical compositions can be administered systemically by oral or parenteral routes. Non-limiting parenteral routes of administration
15 include subcutaneous, intramuscular, intraperitoneal, intravenous, transdermal, inhalation, intranasal, intra-arterial, intrathecal, enteral, sublingual, or rectal. Due to the labile nature of the amino acid sequences parenteral administration is preferred. Preferred modes of administration include aerosols for nasal or bronchial absorption; suspensions for
20 intravenous, intramuscular, intrasternal or subcutaneous, injection; and compounds for oral administration.

Intravenous administration, for example, can be performed by injection of a unit dose. The term "unit dose" when used in reference to a pharmaceutical composition of the present invention refers to physically
25 discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., liquid used to dilute a concentrated or pure substance (either liquid or solid), making that substance the correct (diluted) concentration for use. For injectable
30 administration, the composition is in sterile solution or suspension or may be emulsified in pharmaceutically- and physiologically-acceptable aqueous or

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oleaginous vehicles, which may contain preservatives, stabilizers, and material for rendering the solution or suspension isotonic with body fluids (i.e., blood) of the recipient.

Excipients suitable for use are water, phosphate buffered saline, pH
5 7.4, 0.15 M aqueous sodium chloride solution, dextrose, glycerol, dilute ethanol, and the like, and mixtures thereof. Illustrative stabilizers are polyethylene glycol, proteins, saccharides, amino acids, inorganic acids, and organic acids, which may be used either on their own or as admixtures. The amounts or quantities, as well as routes of administration, used are
10 determined on an individual basis, and correspond to the amounts used in similar types of applications or indications known to those of skill in the art.

Pharmaceutical compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be
15 treated, capacity of the subject's immune system to utilize the active ingredient, and degree of modulation of IR or IGF-1R activity desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are specific for each individual. However, suitable dosages may range from about 10 to 200 nmol active
20 peptide per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous
25 infusions sufficient to maintain picomolar concentrations (e.g., approximately 1 pM to approximately 10 nM) in the blood are contemplated. An exemplary formulation comprises the IR or IGF-1R agonist or antagonist peptide in a mixture with sodium busulfite USP (3.2 mg/ml); disodium edetate USP (0.1 mg/ml); and water for injection q.s.a.d. (1 ml).

30 Further guidance in preparing pharmaceutical formulations can be found in, e.g., Gilman *et al.* (eds), 1990, *Goodman and Gilman's: The*

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Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press; and *Remington's Pharmaceutical Sciences*, 17th ed., 1990, Mack Publishing Co., Easton, PA; Avis *et al.* (eds), 1993, *Pharmaceutical Dosage Forms: Parenteral Medications*, Dekker, New York; Lieberman *et al.* (eds), 1990,
5 *Pharmaceutical Dosage Forms: Disperse Systems*, Dekker, New York.

The present invention further contemplates compositions comprising an IR or IGF-1R agonist or antagonist peptide, and a physiologically acceptable carrier, excipient, or diluent as described in detail herein.

The constructs as described herein may also be used in gene
10 transfer and gene therapy methods to allow the expression of one or more amino acid sequences of the present invention. The amino acid sequences of the present invention can be used for gene therapy and thereby provide an alternative method of treating diabetes which does not rely on the administration or expression of insulin. Expressing insulin for use in gene
15 therapy requires the expression of a precursor product, which must then undergo processing including cleavage and disulfide bond formation to form the active product. The amino acid sequences of this invention, which possess activity, are relatively small, and thus do not require the complex processing steps to become active. Accordingly, these sequences provide a
20 more suitable product for gene therapy.

Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and non-viral transfer methods. A number of viruses have been used as gene transfer vectors, including polyoma, *i.e.*, SV40 (Madzak *et al.*, 1992, *J. Gen. Virol.*, **73**:1533-1536), adenovirus (Berkner, 1992, *Curr. Top. Microbiol. Immunol.*, **158**:39-6; Berkner *et al.*, 1988, *Bio Techniques*, **6**:616-629; Gorziglia *et al.*, 1992, *J. Virol.*, **66**:4407-4412; Quantin *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, **89**:2581-2584; Rosenfeld *et al.*, 1992, *Cell*, **68**:143-155; Wilkinson *et al.*, 1992, *Nucl. Acids Res.*, **20**:2233-2239; Stratford-
30 Perricaudet *et al.*, 1990, *Hum. Gene Ther.*, **1**:241-256), vaccinia virus (Mackett *et al.*, 1992, *Biotechnology*, **24**:495- 499), adeno-associated virus

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(Muzyczka, 1992, *Curr. Top. Microbiol. Immunol.* **158**:91- 123; Ohi et al., 1990, *Gene*, **89**:279-282), herpes viruses including HSV and EBV (Margolskee, 1992, *Curr. Top. Microbiol. Immunol.* **158**:67-90; Johnson et al., 1992, *J. Virol.*, **66**:2952-2965; Fink et al., 1992, *Hum. Gene Ther.* **3**:11-
5 19; Breakfield et al., 1987, *Mol. Neurobiol.*, **1**:337-371; Fresse et al., 1990, *Biochem. Pharmacol.* **40**:2189-2199), and retroviruses of avian (Brandyopadhyay et al., 1984, *Mol. Cell Biol.*, **4**:749-754; Petropoulos et al., 1992, *J. Virol.*, **66**:3391-3397), murine (Miller, 1992, *Curr. Top. Microbiol. Immunol.* **158**:1-24; Miller et al., 1985, *Mol. Cell Biol.*, **5**:431-437; Sorge et al., 1984, *Mol. Cell Biol.*, **4**:1730-1737; Mann et al., 1985, *J. Virol.*, **54**:401-
10 407), and human origin (Page et al., 1990, *J. Virol.*, **64**:5370-5276; Buchschalcher et al., 1992, *J. Virol.*, **66**:2731-2739). Most human gene therapy protocols have been based on disabled murine retroviruses.

Non-viral gene transfer methods known in the art include chemical
15 techniques such as calcium phosphate coprecipitation (Graham et al., 1973, *Virology*, **52**:456-467; Pellicer et al., 1980, *Science*, **209**:1414-1422), mechanical techniques, for example microinjection (Anderson et al., 1980, *Proc. Natl. Acad. Sci. USA*, **77**:5399-5403; Gordon et al., 1980, *Proc. Natl. Acad. Sci. USA*, **77**:7380-7384; Brinster et al., 1981, *Cell*, **27**:223-231;
20 Constantini et al., 1981, *Nature*, **294**:92-94), membrane fusion-mediated transfer via liposomes (Felgner et al., 1987, *Proc. Natl. Acad. Sci. USA*, **84**:7413-7417; Wang et al., 1989, *Biochemistry*, **28**:9508-9514; Kaneda et al., 1989, *J. Biol. Chem.*, **264**:12126-12129; Stewart et al., 1992, *Hum. Gene Ther.* **3**:267-275; Nabel et al., 1990, *Science*, **249**:1285-1288; Lim et al.,
25 1992, *Circulation*, **83**:2007-2011; U.S. Patent Nos. 5,283,185 and 5,795,587), and direct DNA uptake and receptor-mediated DNA transfer (Wolff et al., 1990, *Science*, **247**:1465-1468; Wu et al., 1991, *BioTechniques*, **11**:474-485; Zenke et al., 1990, *Proc. Natl. Acad. Sci. USA*, **87**:3655-3659; Wu et al., 1989, *J. Biol. Chem.*, **264**:16985-16987; Wolff et al.,
30 al., 1991, *BioTechniques*, **11**:474-485; Wagner et al., 1991, *Proc. Natl. Acad. Sci. USA*, **88**:4255-4259; Cotten et al., 1990, *Proc. Natl. Acad. Sci.*

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USA, 87:4033-4037; Curiel *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, 88:8850-8854; Curiel *et al.*, 1991, *Hum. Gene Ther.* 3:147-154).

Many types of cells and cell lines (e.g., primary cell lines or established cell lines) and tissues are capable of being stably transfected by or receiving the constructs of the invention. Examples of cells that may be used include, but are not limited to, stem cells, B lymphocytes, T lymphocytes, macrophages, other white blood lymphocytes (e.g., myelocytes, macrophages, or monocytes), immune system cells of different developmental stages, erythroid lineage cells, pancreatic cells, lung cells, muscle cells, liver cells, fat cells, neuronal cells, glial cells, other brain cells, transformed cells of various cell lineages corresponding to normal cell counterparts (e.g., K562, HEL, HL60, and MEL cells), and established or otherwise transformed cells lines derived from all of the foregoing. In addition, the constructs of the present invention may be transferred by various means directly into tissues, where they would stably integrate into the cells comprising the tissues. Further, the constructs containing the DNA sequences of the peptides of the invention can be introduced into primary cells at various stages of development, including the embryonic and fetal stages, so as to effect gene therapy at early stages of development.

In one approach, plasmid DNA is complexed with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector permits efficient binding, internalization, and degradation of the endosome before the coupled DNA is damaged.

In another approach, liposome/DNA is used to mediate direct *in vivo* gene transfer. While in standard liposome preparations the gene transfer process is non-specific, localized *in vivo* uptake and expression have been reported in tumor deposits, for example, following direct *in situ* administration (Nabel, 1992, *Hum. Gene Ther.* 3:399-410).

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Suitable gene transfer vectors possess a promoter sequence, preferably a promoter that is cell-specific and placed upstream of the sequence to be expressed. The vectors may also contain, optionally, one or more expressible marker genes for expression as an indication of successful
5 transfection and expression of the nucleic acid sequences contained in the vector. In addition, vectors can be optimized to minimize undesired immunogenicity and maximize long-term expression of the desired gene product(s) (see Nabe, 1999, *Proc. Natl. Acad. Sci. USA* **96**:324-326). Moreover, vectors can be chosen based on cell-type that is targeted for
10 treatment.

Illustrative examples of vehicles or vector constructs for transfection or infection of the host cells include replication-defective viral vectors, DNA virus or RNA virus (retrovirus) vectors, such as adenovirus, herpes simplex virus and adeno-associated viral vectors. Adeno-associated virus vectors
15 are single stranded and allow the efficient delivery of multiple copies of nucleic acid to the cell's nucleus. Preferred are adenovirus vectors. The vectors will normally be substantially free of any prokaryotic DNA and may comprise a number of different functional nucleic acid sequences. An example of such functional sequences may be a DNA region comprising
20 transcriptional and translational initiation and termination regulatory sequences, including promoters (e.g., strong promoters, inducible promoters, and the like) and enhancers which are active in the host cells. Also included as part of the functional sequences is an open reading frame (polynucleotide sequence) encoding a protein of interest. Flanking
25 sequences may also be included for site-directed integration. In some situations, the 5'-flanking sequence will allow homologous recombination, thus changing the nature of the transcriptional initiation region, so as to provide for inducible or non-inducible transcription to increase or decrease the level of transcription, as an example.

30 In general, the encoded and expressed peptide may be intracellular, i.e., retained in the cytoplasm, nucleus, or in an organelle, or may be

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secreted by the cell. For secretion, a signal sequence may be fused to the peptide sequence. As previously mentioned, a marker may be present for selection of cells containing the vector construct. The marker may be an inducible or non-inducible gene and will generally allow for positive selection under induction, or without induction, respectively. Examples of marker genes include neomycin, dihydrofolate reductase, glutamine synthetase, and the like. The vector employed will generally also include an origin of replication and other genes that are necessary for replication in the host cells, as routinely employed by those having skill in the art. As an example, the replication system comprising the origin of replication and any proteins associated with replication encoded by a particular virus may be included as part of the construct. The replication system must be selected so that the genes encoding products necessary for replication do not ultimately transform the cells. Such replication systems are represented by replication-defective adenovirus (see G. Acsadi *et al.*, 1994, *Hum. Mol. Genet.* **3**:579-584) and by Epstein-Barr virus. Examples of replication defective vectors, particularly, retroviral vectors that are replication defective, are BAG, (see Price *et al.*, 1987, *Proc. Natl. Acad. Sci. USA*, **84**:156; Sanes *et al.*, 1986, *EMBO J.*, **5**:3133). It will be understood that the final gene construct may contain one or more genes of interest, for example, a gene encoding a bioactive metabolic molecule. In addition, cDNA, synthetically produced DNA or chromosomal DNA may be employed utilizing methods and protocols known and practiced by those having skill in the art.

According to one approach for gene therapy, a vector encoding an IR or IGF-1R agonist or antagonist peptide is directly injected into the recipient cells (*in vivo* gene therapy). Alternatively, cells from the intended recipients are explanted, genetically modified to encode an IR or IGF-1R agonist or antagonist peptide, and reimplanted into the donor (*ex vivo* gene therapy). An *ex vivo* approach provides the advantage of efficient viral gene transfer, which is superior to *in vivo* gene transfer approaches. In accordance with *ex*

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vivo gene therapy, the host cells are first transfected with engineered vectors containing at least one gene encoding an IR or IGF-1R agonist or antagonist peptide, suspended in a physiologically acceptable carrier or excipient such as saline or phosphate buffered saline, and the like, and then
5 administered to the host or host cells. The desired gene product is expressed by the injected cells, which thus introduce the gene product into the host. The introduced gene products can thereby be utilized to treat or ameliorate a disorder that is related to altered insulin or IGF-1 levels (e.g., diabetes).

10 The described constructs may be administered in the form of a pharmaceutical preparation or composition containing a pharmaceutically acceptable carrier and a physiological excipient, in which preparation the vector may be a viral vector construct, or the like, to target the cells, tissues, or organs of the recipient organism of interest, including human and non-
15 human mammals. The composition may be formed by dispersing the components in a suitable pharmaceutically acceptable liquid or solution such as sterile physiological saline or other injectable aqueous liquids. The amounts of the components to be used in such compositions may be routinely determined by those having skill in the art. The compositions may
20 be administered by parenteral routes of injection, including subcutaneous, intravenous, intramuscular, and intrasternal.

J. Cancer Therapeutics

In recent experiments, embryo fibroblasts from IGF-1R knock-out mice have been shown to be highly resistant to transformation by
25 oncogenes such as SV40 T antigen, activated Ha-ras, activated Src, and others (B. Valentinis and R. Baserga, 2001, *Mol. Pathol.*, **54**:133-137). This suggested that IGF-1R was required to mediate malignant transformation by these oncogenes. In addition, IGF-1 and IGF-1R have been shown to act as transforming factors in various forms of human cancer (see above). IGF-1
30 and IGF-2 have also been implicated as factors in the malignant

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transformation of several tissues. Transgenic mice that express a truncated form of IGF-1 that has a decreased affinity for IGFBPs (des(1-3) IGF-1I), show increased incidence of mammary tumors (Hadsell *et al.*, 2000, *Oncogene* **19**:889-898). In addition, mice over-expressing IGF-1I in
5 mammary glands showed increased mammary tumor formation (Bates *et al.*, 1995, *Br. J. Cancer* **72**:1189-1193). Transgenic mice that overexpress IGF-1 in the basal layer of the skin show hyperplasia of the epidermis and increased promotion of spontaneous tumors (DiGiovanni *et al.*, 2000, *Cancer Res.* **60**:1561-1570).

10 IGF-1R also appears to cross-talk with other hormone receptors. Considerable evidence suggests that estrogen can act to increase expression of IGF-1R. This is of particular importance in breast cancer, where the expression of IGF-1R correlates with expression of the estrogen receptor (ER). IGF-1R expression is higher in tumors from ER positive
15 patients. Accordingly, IGF-1R expression could be used as a prognostic marker for breast cancer patients. In addition, high levels of IRS-1, a key intermediate in the IGF-1R signal transduction cascade, correlates with tumor size and shorter disease-free survival in patients with ER positive tumors (D. Sachdev and D. Yee, 2001, *Endocr. Relat. Cancer* **8**:197-209).
20 In addition, treatment with anti-estrogens has been shown to decrease the expression of IGF-1R and IRS-1 (Chan *et al.*, 2001, *Clin. Cancer Res.* **7**:2545-2554). Thus, the cross-talk between IGF-1R and ER may be complex. Yet, it is clear that IGF-signaling promotes malignant transformation in mammary glands. Interestingly, ER positive MCF-7 cells
25 treated with IGF-1 show a sustained activation of the PI3K-Akt pathway and protection against apoptosis induced by serum deprivation. In contrast, ER negative MDA-MB 231 cells show only a transient activation of PI3K-Akt pathway (Bartucci *et al.*, 2001, *Cancer Res.* **61**:6747-6754).

30 Studies have also revealed a connection between IGF-1R-mediated signaling and epidermal growth factor (EGF)-induced signaling through ErbB-receptors. IGF-1R and ErbB-2 (Neu/Her2) have been observed to

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form hetero-oligomers induced by stimulation with heregulin or IGF-1 (Balana *et al.*, 2001, *Oncogene*, **19**:34-47, 2001). In glioblastomas, resistance to a chemical inhibitor of the ErbB receptor tyrosine kinase has been correlated with increased IGF-1R expression and constitutive PI3K signaling (Chakravarti *et al.*, 2002, *Cancer Res.* **62**:200-207). In breast cancer cell lines over expressing ErbB-2, increased IGF-1R signaling was observed in the presence of the anti-ErbB-2 receptor monoclonal antibody Herceptin®/trastuzumab (Lu *et al.*, 2001, *J. Natl. Cancer Inst.* **93**:1852-1857).

Modulation of IGF-signaling in various malignant cells has provided further evidence for the involvement of the IGF-1R in cancer. Abrogation of IGF-1R expression by antisense RNA reversed the transformed phenotype in cervical cancer cells. Antisense to IGF-1R also inhibited glioblastoma and melanoma xenografts in nude mice (Resnicoff *et al.*, 1994, *Cancer Res.* **54**:4848-4850; Resnicoff *et al.*, 1994, *Cancer Res.* **54**:2218-2222; Nakamura *et al.*, 2000, *Cancer Res.* **60**:760-765, 2000). Experiments have also indicated that IGF-1R is involved in the development and maintenance of metastatic phenotypes. In particular, high expression of a dominant negative mutant of IGF-1R (486stop) in ER positive breast cancer cells has been shown to inhibit adhesion, invasion, and metastasis of the cells (Dunn *et al.*, 1998, *Cancer Res.* **58**:3353-3361). Moreover, lung carcinoma cells exhibited an enhanced metastatic phenotype following overexpression of IGF-1R (Long *et al.*, 1998, *Exp. Cell Res.* **238**:116-121). In addition, activation of IGF-1R has been shown to block apoptotic pathways. Apoptosis in mammary glands was inhibited in IGF-1 transgenic mice (Hadsell *et al.*, 2000, *Oncogene* **19**:889-898). Moreover, down-regulation of IGF-1R function, either by antisense strategies or dominant negative mutants, caused massive apoptosis of tumor cells *in vitro* and *in vivo*. IGF-1 has also been shown to inhibit apoptosis associated with transformation by the c-myc oncogene and apoptosis induced by chemotherapeutic agents. The anti-apoptotic signaling of IGF-1 has been attributed to the PI3K-Akt

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pathway, although other pathways may mediate similar effects (Butt *et al.*, 1999, *Immunol. Cell Biol.* **77**:256-262; B. Valentinis and R. Baserga, 2001, *Mol. Pathol.* **54**:133-137).

5 The sum of these observations indicate the importance of identifying antagonists or inhibitors of IGF-1 and/or IGF-1R. Attempts have been made to develop clinically relevant inhibitors of IGF-1R using monoclonal antibodies, antisense strategies, and peptide fragments derived from the natural ligand (Dunn *et al.*, 1998, *Cancer Res.* **58**:3353-3361; Z. Pietrzkowski *et al.*, 1992, *Cancer Res.* **52**:6447-6451; Z. Pietrzkowski *et al.*, 10 1993, *Cancer Res.* **53**:1102-1106; Rubini *et al.*, 1999, *Exp. Cell Res.* **251**:22-32). Using an alternate approach, this invention provides methods, kits, and compositions (e.g., pharmaceutical compositions) comprising IGF-1R antagonist peptides, or small molecule mimetics thereof, that can be useful in the diagnosis, treatment, and monitoring of one or more cancers. 15 In some cases, the compositions, methods, and kits of the invention can also be used to determine the prognosis of a IGF-related medical condition (e.g., cancer). Advantageously, certain IGF-1R antagonist peptides disclosed herein are specific for Site 1 or Site 2 of the IGF-1 receptor.

In accordance with the invention, non-limiting cancer types include 20 carcinoma, sarcoma, myeloma, leukemia, and lymphoma, and mixed types of cancers, such as adenosquamous carcinoma, mixed mesodermal tumor, carcinosarcoma, and teratocarcinoma. Representative cancers include, but are not limited to, bladder cancer, lung cancer, breast cancer, colon cancer, rectal cancer, endometrial cancer, ovarian cancer, head and neck cancer, 25 prostate cancer, and melanoma. Specifically included are AIDS-related cancers (e.g., Kaposi's Sarcoma, AIDS-related lymphoma), bone cancers (e.g., osteosarcoma, malignant fibrous histiocytoma of bone, Ewing's Sarcoma, and related cancers), and hematologic/blood cancers (e.g., adult acute lymphoblastic leukemia, childhood acute lymphoblastic leukemia, 30 adult acute myeloid leukemia, childhood acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia,

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cutaneous T-cell lymphoma, adult Hodgkin's disease, childhood Hodgkin's disease, Hodgkin's disease during pregnancy, mycosis fungoides, adult non-Hodgkin's lymphoma, childhood non-Hodgkin's lymphoma, non-Hodgkin's lymphoma during pregnancy, primary central nervous system lymphoma,
5 Sezary syndrome, cutaneous T-cell lymphoma, Waldenström's macroglobulinemia, multiple myeloma/plasma cell neoplasm, myelodysplastic syndrome, and myeloproliferative disorders).

Also included are brain cancers (e.g., adult brain tumor, childhood brain stem glioma, childhood cerebellar astrocytoma, childhood cerebral
10 astrocytoma, childhood ependymoma, childhood medulloblastoma, supratentorial primitive neuroectodermal and pineal, and childhood visual pathway and hypothalamic glioma), digestive/gastrointestinal cancers (e.g., anal cancer, extrahepatic bile duct cancer, gastrointestinal carcinoid tumor, colon cancer, esophageal cancer, gallbladder cancer, adult primary liver
15 cancer, childhood liver cancer, pancreatic cancer, rectal cancer, small intestine cancer, and gastric cancer), musculoskeletal cancers (e.g., childhood rhabdomyosarcoma, adult soft tissue sarcoma, childhood soft tissue sarcoma, and uterine sarcoma), and endocrine cancers (e.g., adrenocortical carcinoma, gastrointestinal carcinoid tumor, islet cell
20 carcinoma (endocrine pancreas), parathyroid cancer, pheochromocytoma, pituitary tumor, and thyroid cancer).

Further included are neurologic cancers (e.g., neuroblastoma, pituitary tumor, and primary central nervous system lymphoma), eye cancers (e.g., intraocular melanoma and retinoblastoma), genitourinary
25 cancers (e.g., bladder cancer, kidney (renal cell) cancer, penile cancer, transitional cell renal pelvis and ureter cancer, testicular cancer, urethral cancer, Wilms' tumor and other childhood kidney tumors), respiratory/thoracic cancers (e.g., non-small cell lung cancer, small cell lung cancer, malignant mesothelioma, and malignant thymoma), germ cell
30 cancers (e.g., childhood extracranial germ cell tumor and extragonadal germ cell tumor), skin cancers (e.g., melanoma, and merkel cell carcinoma),

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gynecologic cancers (e.g., cervical cancer, endometrial cancer, gestational trophoblastic tumor, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, uterine sarcoma, vaginal cancer, and vulvar cancer), and unknown primary cancers.

- 5 Specific breast cancers include, but are not limited to, non-invasive cancers, such as ductal carcinoma *in situ* (DCIS), intraductal carcinoma lobular carcinoma *in situ* (LCIS), papillary carcinoma, and comedocarcinoma, or invasive cancers, such as adenocarcinomas, or carcinomas, e.g., infiltrating ductal carcinoma, infiltrating lobular carcinoma, 10 infiltrating ductal and lobular carcinoma, medullary carcinoma, mucinous (colloid) carcinoma, comedocarcinoma, Paget's Disease, papillary carcinoma, tubular carcinoma, and inflammatory carcinoma. Specific prostate cancers may include adenocarcinomas and sarcomas, or pre-cancerous conditions, such as prostate intraepithelial neoplasia (PIN).
- 15 Specific lung cancers include those relating to tumors such as bronchial carcinoid (bronchial adenoma), chondromatous hamartoma (benign), solitary lymphoma, and sarcoma (malignant) tumors, as well as lung cancers relating to multifocal lymphomas. Bronchogenic carcinomas may present as squamous cell carcinomas, small cell carcinomas, non-small cell 20 carcinomas, or adenocarcinomas.

 The IGF-1R antagonist peptides of the invention may be administered individually, or in combination with other IGF-1 or IGF-1R antagonists or inhibitors. Alternatively, the disclosed IGF-1R antagonist peptides can be used in combination with other cancer therapies, e.g., surgery, radiation, 25 biological response modification, immunotherapy, hormone therapy, and/or chemotherapy. For prostate cancers, non-limiting examples of chemotherapeutic agents include docetaxel, paclitaxel, estramustine, etoposide, vinblastine, mitoxantrone, and paclitaxel. For breast cancers, non-limiting examples of chemotherapeutic and biological agents include 30 cyclophosphamide, methotrexate, 5-fluorouracil, doxorubicin, tamoxifen, paclitaxel, docetaxel, navelbine, capecitabine, mitomycin C, Interferons,

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interleukin-2, lymphocyte-activated killer cells, tumor necrosis factors, and monoclonal antibodies (e.g., mAb to HER-2/neu receptor (trastuzumab) Herceptin®). For lung cancers, non-limiting examples of chemotherapeutic and biological agents include, but are not limited to, platinum compounds
5 (e.g., cisplatin or carboplatin), vinca alkaloids (e.g., vinorelbine, vincristine, or vinblastine), taxines (e.g., docetaxel or paclitaxel), and various topoisomerase inhibitors.

EXAMPLES

The examples as set forth herein are meant to exemplify the various
10 aspects of the present invention and are not intended to limit the invention in any way.

The following materials were used in the examples described below. Soluble IGF-1R was obtained from R&D Systems (Minneapolis, MN; Cat. # 391-GR/CF). Insulin receptor was prepared according to Bass *et al.*, 1996.
15 The insulin was either from Sigma (St. Louis, MO; Cat. # I-0259) or Boehringer. The IGF-1 was from PeproTech (Cat. # 100-11). All synthetic peptides were synthesized by Novo Nordisk, AnaSpec, Inc. (San Jose, CA), PeptioGenics (Livermore, CA), or Research Genetics (Huntsville, AL) at >80% purity. The Maxisorb Plates were from NUNC via Fisher (Cat. #
20 12565347). The HRP/Anti-M13 conjugate was from Pharmacia (Cat. # 27-9421-01). The ABTS solution was from BioF/X (Cat. # ABTS-0100-04).

Example 1: Monomer and Dimer Peptides

A. Cloning

Monomer and dimer peptides were constructed and expressed as
25 protein fusions to a chitin binding domain (CBD) using the pTYB2 vector from the IMPACT™-CN system (New England Biolabs (NEB), Beverly, MA). The pTYB2 vector encodes a protein-splicing element (termed intein), which

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initiates self-cleavage upon the addition of DTT. The intein self-cleavage separates the dimer from the affinity tag, to allow purification.

In the pTYB2 construct, the C-terminus of the peptide sequence was fused to the N-terminus of the intein/CBD sequence. Two peptide-flanking
5 epitope tags were included: a shortened-FLAG® at the N-terminus and E-Tag at the C-terminus. This fusion was generated by ligating a vector fragment encoding the intein/CBD with a PCR product encoding the peptide of interest.

The vector fragment was obtained by digesting at appropriate
10 restriction sites the pTBY2 vector. The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN, Valencia, CA). To obtain the PCR product of the target proteins, primers were synthesized which anneal to appropriate sequences. The vector and insert were ligated overnight at 15°C. The ligation product was purified
15 using QIAquick spin columns (QIAGEN) and electroporations were performed at 1500 V in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 µl of *E. coli* strain BL21.

Immediately following electroporation, 1 ml of pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added to the
20 transformants. The transformants were grown at 37°C for 1 h, and then plated onto 2xYT-AG plates and incubated overnight at 37°C. Individual colonies were isolated and used to inoculate 2xYT-G. The cultures were grown overnight at 37°C. Plasmid DNA was isolated from the cultures and sequencing was performed to confirm that the correct construct was
25 obtained.

B. Small-scale expression of peptide-CBD fusion proteins

E. coli ER2566 (New England Biolabs) containing plasmids encoding peptide-CBD fusion proteins were grown in 2xYT-AG at 37°C overnight, with agitation (250 rpm). The following day, the cultures were used to inoculate
30 media (2x YT-G) to obtain an OD₆₀₀ of 0.1. Upon reaching an OD₆₀₀ of 0.6,

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expression of the fusion protein was induced by the addition of IPTG (isopropyl- β -D-thiogalactopyranoside) to a final concentration of 0.3 mM. Cells were grown for 3 h. Following this, cells were pelleted by centrifugation and the cell pellets were analyzed by SDS-PAGE
5 electrophoresis. Production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Amersham Pharmacia).

C. Large-scale expression and purification of soluble peptide-CBD fusion proteins

10 *E. coli* ER2566 carrying plasmids encoding the fusion proteins were grown in 2xYT-AG media at 37°C for 8 h, with agitation (250 rpm). The cultures were back-diluted into to 2 L volumes of 2xYT-A to achieve an OD₆₀₀ of 0.1. Upon reaching an OD₆₀₀ of 0.5, IPTG was added to a final concentration of 0.3 mM. Cells were grown at 30°C overnight. The next day
15 cells were isolated by centrifugation. Samples of the cell pellet were analyzed by SDS-PAGE followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

D. Purification

20 The cell pellets were disrupted mechanically by sonication or chemically by treatment with the mild detergent. After removal of cell debris by centrifugation, the soluble proteins in the clarified lysate were prepared for chromatographic purification by dilution or dialysis into the appropriate starting buffer. The CBD fusions were purified by chitin affinity
25 chromatography according to the manufacturer's instructions (New England Biolabs). The lysate was loaded onto a chitin affinity column and the column was washed with 10 volumes of column buffer. Three bed volumes of the DTT containing cleavage buffer were loaded onto the column and the column was incubated overnight. The next day, the target protein was

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eluted by continuing the flow of the cleavage buffer without DTT. The purified proteins were analyzed for purity and integrity by SDS-PAGE and Western blot analysis according to standard protocols.

Example 2: PEG-Based Dimer Peptides

5 A. Synthesis of the aldehyde containing peptide

The peptide was synthesized by stepwise solid phase synthesis on Rink amide Tentagel (0.21 mmol/g). Three equivalents of Fmoc-amino acids were used. The serine residue was introduced into the peptide by either coupling Fmoc-Ser(tBu)-OH to the N-terminal peptide or coupling
10 Boc-Ser(tBu) to a selectively protected lysine side-chain. The peptide was then deprotected and cleaved from the resin by treatment with 95% TFA (trifluoroacetic acid; aq) containing TIS (triisopropylsilan). Periodate oxidation, using 2 equivalent of NaIO₄ in 20% DMSO (dimethyl sulfoxide)-80% phosphate buffer pH 7.5 (45 µl/µmol peptide) for 5 min at room
15 temperature (RT), converted the 2-amino alcohol moiety in an α-oxoacyl group. The peptide was purified immediately following oxidation.

B. Synthesis of the PEG-based dimer

The unprotected and oxidized peptide (4.2 equivalent) was dimerized on the dioxyamino-PEG (polyethylene glycol)-linker (1 equivalent) in 90%
20 DMSO-10% 20 mM NaOAc buffer, pH 5.1 (4.2 µl/µmol peptide). The solution was left for 1 h at 38°C and the progress of the reaction was monitored by MALDI-MS (matrix-assisted laser desorption/ionization mass spectrometry). Following this, the crude dimer was purified by semi-preparative HPLC (high performance liquid chromatography).



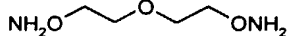
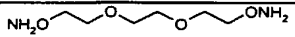
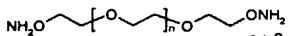

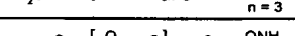
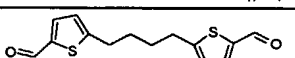
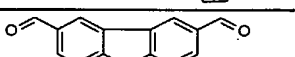
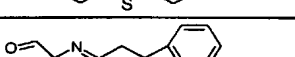
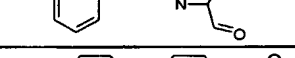
25 The molecular weights and inter peptide distance of various linkers is shown in Table 3, below.

TABLE 3

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Structure	Number	MW	MW (- 2H ₂ O)
	1	100.1	64.1
	2	58.04	22.04
	3	149.15	113.15
	4	150.14	114.14
	5	134.13	98.13
	6	134.13	98.13
	7	134.13	98.13
	8	234.25	198.25
	9	302.3	266.3
	10	72.06	36.06
	11	86.09	50.09
	12	114.14	78.14

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	13	128.08	92.08
	14	142.19	106.19
(HCO)₄-(Lys)₂-Lys-Gly-NH₂	15		
	16	136.2	100.2
	17	180.2	144.2
	18	224.3	188.3
	19	268.3	232.3
	20	312.4	276.4
	21	278.4	242.4
	22	240.3	204.3
	23	240.3	204.3
	24	210.2	192.2

Example 3: Determination of Insulin Receptor Binding

IR was incubated with ¹²⁵I-labeled insulin at various concentrations of test substance and the K_d was calculated. According to this method, human insulin receptor (HIR) or human IGF-1 receptor (HIGF-1R) was purified from transfected cells after solubilization with Triton X-100. The assay buffer contained 100 mM HEPES (pH 7.8), 100 mM NaCl, 10 mM MgCl₂, 0.5% human serum albumin (HSA), 0.2% gammaglobulin and 0.025% Triton X-100. The receptor concentration was chosen to give 30-60% binding of 2000 cpm (3 pM) of its ¹²⁵I-labeled ligand (TyrA14-¹²⁵I-HI or Tyr31-¹²⁵I-IGF1) and a dilution series of the substance to be tested was added. After equilibration for 2 days at 4°C, each sample (200 μl) was precipitated by addition of 400 μl 25% PEG 6000, centrifuged, washed with 1 ml 15% PEG 6000, and counted in a gamma-counter.

The insulin/IGF-1 competition curve was fitted to a one-site binding model and the calculated parameters for receptor concentration, insulin

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affinity, and non-specific binding were used in calculating the binding constants of the test substances. Representative curves for insulin competition are shown in Figures 10A-10C; 11A-11D. Qualitative data are provided in Table 4, below.

- 5 Table 4 illustrates IR affinities for the RP9 monomer peptide and various RP9 monomer truncations. The results demonstrate that RP9 N-terminal sequence (GSLD; SEQ ID NO:1785) and C-terminal sequence (LGKK; SEQ ID NO:1786) can be deleted without substantially affecting HIR binding affinity (Table 4).

10

TABLE 4

Peptide	SEQ ID NO:	Formula	Site IR	Sequence	HIR Kd (mol/l)
S386	1559	1	1	GSLDESFYDWFERQLG	$3.2 \cdot 10^{-7}$
S395	1787	1	1	GSLDESFYDWFERQL	$9.1 \cdot 10^{-8}$
S394	1788	1	1	GSLDESFYDWFERQ	$8.1 \cdot 10^{-8}$
S396	1789	1	1	GSLDESFYDWFER	$>2 \cdot 10^{-5}$
S399	1790	1	1	ESFYDWFERQL	$9.1 \cdot 10^{-8}$
S400	1791	1	1	ESFYDWFERQ	$6.3 \cdot 10^{-7}$

- Figures 10A-10C demonstrate that Site 1-Site 2 heterodimer peptides 537, 538, and 539 bound to IR with substantially higher (several orders of magnitude) affinity than corresponding monomer (D117 and 540) and homodimer (521 and 535) peptides. Figures 11A-11D demonstrate that Site 1-Site 2 heterodimer peptides, 537 and 538, bound to IR with markedly higher affinity than the monomer peptide D117.

Example 4: Adipocyte Assay for Determination of Insulin Agonist Activity

- 20 Insulin increases uptake of ^3H glucose into adipocytes and its conversion into lipid. Incorporation of ^3H into the lipid phase was determined by partitioning of lipid phase into a scintillant mixture, which excludes water-soluble ^3H products. The effect of compounds on the incorporation of ^3H glucose at a sub-maximal insulin dose was determined, and the results

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expressed as increase relative to full insulin response. The method was adapted from Moody *et al.*, 1974, *Horm. Metab. Res.* 6(1):12-6.

Mouse epididymal fat pads were dissected out, minced into digestion buffer (Krebs-Ringer 25 mM HEPES, 4% HSA, 1.1 mM glucose, 0.4 mg/ml
5 Collagenase Type 1, pH 7.4), and digested for up to 1.5 h at 36.5°C. After filtration, washing (Krebs-Ringer HEPES, 1% HSA), and resuspension in assay buffer (Krebs-Ringer HEPES, 1% HSA), free fat cells were pipetted into 96-well Picoplates (Packard), containing test solution and approximately an ED₂₀ insulin.

10 The assay was started by addition of ³H glucose (Amersham TRK 239), in a final concentration of 0.45 mM glucose. The assay was incubated for 2 h, 36.5°C, in a Labshaker incubation tower, 400 rpm, then terminated by the addition of Permablend/Toluene scintillant (or equivalent), and the plates sealed, before standing for at least 1 h and detection in a Packard
15 Top Counter or equivalent. A full insulin standard curve (8 dose) was run as control on each plate.

Data are presented graphically, as effect of compound on an (approximate) ED₂₀ insulin response, with data normalized to a full insulin response. The assay can also be run at basal or maximal insulin
20 concentration. Representative dose-response curves for insulin and IGF-1 are shown in Figures 12-18. Qualitative data are shown in Tables 5-7.

In free fat cell (FFC) assays, truncated synthetic RP9 monomer peptides S390 and S394 showed potency similar to full-length RP9 monomer peptides (Figures 12A-12D). Truncated synthetic RP9 homodimer
25 peptides S415 and S417 were highly potent in FFC assays, but less potent than full-length RP9 homodimer peptides (Figures 13A-13C; compare to peptides 521 and 535, described below). The potency of recombinant RP9 homodimer peptides 521 and 535 in FFC assays is shown in Figures 14A-14C. The curves are flattened, suggesting that the binding mechanism may
30 not be mediated by simple intramolecular binding (Figures 14A-14C).

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Results further indicated that synthetic RP9 homodimer peptides S337 and S374 showed increased HIR binding affinity and increased potency in FFC assays compared to synthetic RP9 monomer, S371 (Table 5). Similarly, synthetic RP9 homodimer peptides S314 and S317 showed increased HIR binding affinity and increased potency in FFC assays compared to synthetic RP9 monomer, S371, and various RP9 truncations (Table 6).

TABLE 5

Pep.	SEQ ID NO:	Formula	Site IR	Monomer or Dimer	Sequence	HIR K_d (mol/l)	FFC
S371	1558	1	1	M (RP9)	GSLDESFYDWFERQLGKK	$6.3 \cdot 10^{-7}$	+
S337	1792	1-1	1-1	D, C-Term 23	(GSLDESFYDWFERQLGKK-Lig) ₂ -23	$1.1 \cdot 10^{-8}$	+++++
S374	1793	1-1	1-1	D, N-Term 17	17-(GSLDESFYDWFERQLGKK) ₂	$1.8 \cdot 10^{-7}$	++++

10 M = monomer; D = dimer; C-Term = C-terminal linker (C-C); N-Term = N-terminal linker (N-N); 23 and 17 represent specific chemical linkers (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist.

TABLE 6

Peptide	SEQ ID NO:	Form.	Site IR	Mon. or Dimer	Sequence	HIR K_d (mol/l)	FFC
S371 (RP9)	1558	1	1	M	GSLDESFYDWFERQLGKK	$6.3 \cdot 10^{-7}$	+
S395	1787	1	1	M	GSLDESFYDWFERQL	$9.1 \cdot 10^{-8}$	+
S394	1788	1	1	M	GSLDESFYDWFERQ	$8.1 \cdot 10^{-8}$	++
S396	1789	1	1	M	GSLDESFYDWFER	$>2 \cdot 10^{-5}$	0
S390	1794	1	1	M	ESFYDWFERQLG	$6.2 \cdot 10^{-7}$	+
S399	1790	1	1	M	ESFYDWFERQL	$9.1 \cdot 10^{-8}$	++
S400	1791	1	1	M	ESFYDWFERQ	$6.3 \cdot 10^{-7}$	0
S415	1795	1-1	1-1	D; C-Term	(ESFYDWFERQLGK) ₂ -23	$1.0 \cdot 10^{-7}$	++++
S417	1796	1-1	1-1	D; N-Term	23-(ESFYDWFERQLG) ₂	$9.2 \cdot 10^{-7}$	+++

15 M = monomer; D = dimer; C-Term = C-terminal linker (C-C); N-Term = N-terminal linker (N-N); 23 represents a specific chemical linker (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist; Form. = formula; Mon. = monomer;

20 Site 1-Site 2 dimer peptides 537 and 538 were inactive in the FFC assays using the standard concentration of insulin (Figures 15A-15C). However, Site 1-Site 2 dimer peptides 537 and 538 were antagonists in the

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FFC assay in the presence of a stimulating concentration of insulin (Figures 16A-16C). In contrast, Site 2-Site 1 dimer peptide 539 was a full agonist in the FFC assay, with a slope similar to that of insulin (Figures 17A-17B).

Additional experiments confirmed that FFC assay activity of Site 1-Site 2 dimer peptides was affected by the orientation of the monomer subunits (Figures 18A-18D). In particular, dimer peptides comprising Site 1 (S372 or S373) and Site 2 (S451 or S452) monomer subunits exhibited antagonist activity in the Site 1-Site 2 orientation (C-N linkage) (dimer peptide S453); moderate levels of agonist activity in the Site 1-Site 2 orientation (N-N or C-C linkage) (dimer peptides S454 and S456); and high levels of agonist activity in the Site 2-Site 1 orientation (C-N linkage) (dimer peptide S455) (Figures 18A-18D).

Table 7, below, shows the HIR binding affinity and FFC assay potency of various synthetic peptides, including Site 1-Site 1 dimer peptides S325, S329, S332; S333, S334, S335, S336, S337, S349, S350, S351, S352, S353, S354, S361, S362, S363, S374, S375, S376, S378, S379, S380, S381, S414, S415, S416, S417, S418, S420, and S424. These synthetic dimer peptides exhibited properties comparable to dimer peptides 521 and 535, regardless of the orientation of the monomer subunits. In particular, synthetic Site 1-Site 2 dimer peptides S425, S453, and S459 exhibited antagonist properties comparable to those of the Site 1-Site 2 dimer peptides 537 and 538. Synthetic Site 1-Site 2 dimer peptides S455, S457, and S458 exhibited agonist properties comparable to the dimer peptide 539. Synthetic Site 1-Site 2 dimer peptides S436, S437, S438, S454, S456 act as partial agonists in the FFC assay (i.e., the peptides exhibit a maximal response of less than 100% that of insulin), which is shown in the table as “++” and “+++”.

Table 7 also shows properties of truncated monomer and dimer peptides, and thereby indicates which N- or C-terminal residues can be deleted without substantial loss of HIR binding affinity (e.g., see synthetic peptides S386 through S392, S394 through S403, and S436 through S445).

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Notably, certain Site 2-Site 1 dimers show IR affinities of 2×10^{-11} (see, e.g., S519 and S520). These peptides are also very potent in the fat cell assay (Figures 31A-31B) and even more potent in the HIR kinase assay (Figures 32A-32B) (kinase assay described below).

TABLE 7

Peptide	SEQ ID NO:	Formula	Linkage	Site IR	Sequence	HIR Kd (mol/l)	FFC
S105	1797	F1	-	1	FHENFYDWFVRQVAKK	3.1×10^{-7}	++
S106	1798	F1	-	1	FHENFYDWFVRQASKK	4.2×10^{-7}	++
S107	1799	F1	-	1	FHENFYDWFVRAVSKK	10.0×10^{-7}	+
S108	1800	F1	-	1	FHENFYDWFVAQVSKK	7.5×10^{-7}	+
S109	1801	F1	-	1	FHENFYDWFARQVSKK	2.3×10^{-7}	++
S110	1802	F1	-	1	FHEAFYDWFVRQVSKK	2.2×10^{-7}	++
S111	1803	F1	-	1	FHANFYDWFVRQVSKK	3.3×10^{-7}	0
S112	1804	F1	-	1	FAENFYDWFVRQVSKK	6.1×10^{-7}	+
S113	1805	F1	-	1	AHENFYDWFVRQVSKK	5.9×10^{-7}	+
S114	1556	F1	-		fhenfydwfvrqvs	8.3×10^{-6}	0
S115	1806	F1	-	1	EFHENFYDWFVRQVSEE	6.5×10^{-7}	+
S116	1807	F1	-	1	FHENFYDWFVRQVSKK	1.4×10^{-6}	++
S117	1808	F2	-	1	HETFYSMIRSLAK	2.7×10^{-6}	0
S118	1809	F2	-	1	SDGFYNAIELLS	2.4×10^{-6}	+
S119	1810	F2	-	1	SLNFYDALQLLAKK	1.8×10^{-6}	0
S120	1811	F2	-	1	HDPFYSMMKSLK	2.0×10^{-6}	0
S121	1812	F2	-	1	NSFYEARMLSSK	3.1×10^{-6}	0
S122	1813	F7	-		HPTSKEIYAKLLK	9.3×10^{-6}	0
S123	1814	F7	-		HPSTNQMLMKLKF	1.6×10^{-5}	0
S124	1815	F7	-		HPPLSELKFLIKK	2.3×10^{-5}	0
S127	1816	F2	-	1	WSDFYSYFQGLD	1.2×10^{-6}	0
S128	1817 and 1818	F1-F1	C-C	1-1	(FHENFYDWFVRQVSKK) α -Dap	1.1×10^{-6}	++
S129	1819	F2	-	1	SSNFYQALMLLS	2.9×10^{-6}	0

S131	1820	F1	-	1	FHENFYDWFVRQVSKK-Lig	1.2*10 ⁻⁶	+
S137	1821	F1	-	1	HENFYGWFRQVSKK	7.7*10 ⁻⁷	0
S145	1822 and 1823	F1-F1	C-C	1-1	(FHENFYDWFVRQVSKK) ₂ -Lys	1.5*10 ⁻⁶	++
S158	1780	F1	-	1	FHENFYDWFVRQVSK	8.1*10 ⁻⁷	+
S165	1554	F1	-	1	FYDWF	>2*10 ⁻⁵	0
S166	1824	F1	-	1	FYDWFKK	>2*10 ⁻⁵	0
S167	1825	F1	-	1	AFYDWFACK	>2*10 ⁻⁵	-
S168	1826	F1	-	1	AAAFYDWFAAAAKK	3.8*10 ⁻⁶	0
S169	1827 and 1828	F1-F1	N-N	1-1	12-(Lig-FHENFYDWFVRQVSKK) ₂	5.8*10 ⁻⁷	++
S170	1829 and 1830	F1-F1	N-N	1-1	(CGFHENFYDWFVRQVSKK) ₂ (linked at cysteines)	7.0*10 ⁻⁷	+++
S171	1831	F1	-	1	CGFHENFYDWFVRQVSKK	2.9*10 ⁻⁶	+++
S172	1832 and 1833	F1-F1	N-N	1-1	14-(Lig-FHENFYDWFVRQVSKK) ₂	4.8*10 ⁻⁶	+++
S173	1834	F3	-	1	LDALDRLMRYFEERPSL	1.2*10 ⁻⁶	0
S174	1835	F3	-	1	PLAELWAYFEHSEQGRSSAH	1.6*10 ⁻⁵	0
S175	1560	F1	-	1	GRVDWLQRNANFYDWFVAELG	2.3*10 ⁻⁷	+++
S176	1836	F1	-	1	NGVERAGTGDNFYDWFVAQLH	4.7*10 ⁻⁷	+
S177	1837	F2	-	1	EHWNTVDPPFYFTLFEWLRESG	2.7*10 ⁻⁶	0
S178	1838	F2	-	1	EHWNTVDPPFYQYFSELLRESG	1.3*10 ⁻⁷	++
S179	1839	F1	-	1	QSDSGTVHDRFYGWFRDRTWAS	5.4*10 ⁻⁷	+
S180	1840	F1	-	1	AFYDWFACK	>2*10 ⁻⁵	0
S181	1841	F1	-	1	AFYDWFA	>2*10 ⁻⁵	0
S182	1842	F1	-	1	AFYDWF	>2*10 ⁻⁵	0
S183	1843	F1	-	1	FYDWFA	>2*10 ⁻⁵	0
S184	1844	F1	-	1	Ac-FYDWF	>2*10 ⁻⁵	0
S214	1845	F1	-	1	AFYEWFAKK	>2*10 ⁻⁵	0
S215	1846	F1	-	1	AFYGWFAKK	>2*10 ⁻⁵	0
S216	1847	F1	-	1	AFYKWFACK	>2*10 ⁻⁵	0

S217	1848 and 1849	F2-F2	C-C	1-1	(SDGFYNAIELLS-Lig) ₂ -14	3.9*10 ⁻⁸	++
S218	1850 and 1851	F1-F1	C-C	1-1	(AFYDWFVAKK-Lig) ₂ -14	1.1*10 ⁻⁵	0
S219	1852	F1	-	1	FHENAYDWFVRQVSKK	>2*10 ⁻⁵	0
S220	1853	F1	-	1	FHENFADWFVRQVSKK	>2*10 ⁻⁵	0
S221	1854	F1	-	1	FHENFYAWFVRQVSKK	1.1*10 ⁻⁶	+
S222	1855	F1	-	1	FHENFYDAFVRQVSKK	>2*10 ⁻⁵	0
S223	1856	F1	-	1	FHENFYDWA VRQVSKK	>2*10 ⁻⁵	0
S226	1857	F6	-	2	QLEEWAGVQCEVYGRECPs	1.6*10 ⁻⁶	
S227	1858	F1	-	1	CGGFHENFYDWFVRQVSKK	5.1*10 ⁻⁷	++
S228	1859 and 1860	F1-F1	N-N	1-1	(CGGFHENFYDWFVRQVSKK) ₂ (linked at cysteines)	3.6*10 ⁻⁷	++
S229	1861 and 1862	F2-F4	C-C	1-2	SDGFYNAIELLS-Lig 12	4.4*10 ⁻⁹	0
					KHLCVLEELFWGASLFGYCSGKK-Lig		
S231	1863 and 1864	F1-F1	C-C	1-1	(FHENFYDWFVRQVSKKGGG-Lig) ₂ -14	2.7*10 ⁻⁷	+
S232	1865 and 1866	F1-F1	N-N	1-1	14-(Lig-GGGFHENFYDWFVRQVSKK) ₂	3.8*10 ⁻⁷	+++
S233	1867 and 1868	F1-F2	C-C	1-1	FHENFYDWFVRQVSKK-Lig 14	2.6*10 ⁻⁷	+
					SDGFYNAIELLS-Lig		
S234	1869	F1	-	1	RVDWLQRNANFYDWFV AELG	1.3*10 ⁻⁷	++
S235	1870	F1	-	1	VDWLQRNANFYDWFV AELG	5.3*10 ⁻⁸	++
S236	1871	F1	-	1	DWLQRNANFYDWFV AELG	1.0*10 ⁻⁷	++
S237	1872	F1	-	1	WLQRNANFYDWFV AELG	8.5*10 ⁻⁷	0
S238	1873	F1	-	1	LQRNANFYDWFV AELG	8.5*10 ⁻⁷	0
S239	1874	F1	-	1	QRNANFYDWFV AELG	1.3*10 ⁻⁶	0
S240	1875	F1	-	1	RNANFYDWFV AELG	1.4*10 ⁻⁶	
S241	1876	F1	-	1	NANFYDWFV AELG	1.6*10 ⁻⁶	
S242	1877	F1	-	1	ANFYDWFV AELG	2.0*10 ⁻⁶	

S243	1878	F1	-	1	NFYDWFVAELG	2.0*10 ⁻⁶	
S244	1879	F1	-	1	GRVDWLQRNANFYDWFVAELG-Lig	2.2*10 ⁻⁷	++
S245	1880	F1	-	1	Lig-GRVDWLQRNANFYDWFVAELG	2.2*10 ⁻⁷	+
S246	1881 and 1882	F8-F1	C-C	3-1	ACAWPTYWNCGGGG-Lig 14 FHENFYDWFVRQVSKK-Lig	5.0*10 ⁻⁶	
S248	1883	F1	-	1	GRVDWLQRNANFYDWFVAEL	6.3*10 ⁻⁸	++
S249	1884	F1	-	1	GRVDWLQRNANFYDWFVAE	7.4*10 ⁻⁷	0
S250	1885	F1	-	1	GRVDWLQRNANFYDWFVA	8.9*10 ⁻⁶	0
S251	1886	F1	-	1	GRVDWLQRNANFYDWFV	5.6*10 ⁻⁶	
S252	1887 and 1888	F2-F2	C-C	1-1	(SDGFYNAIELLS-Lig) ₂ -14	4.4*10 ⁻⁷	0
S253	1889 and 1890	F1-F1	C-C	1-1	(GRVDWLQRNANFYDWFVAELG-Lig) ₂ -14	2.2*10 ⁻⁸	++
S255	1891 and 1892	F2-F2	C-C	1-1	(SDGFYNAIELLSGGG-Lig) ₂ -14	1.6*10 ⁻⁶	0
S256	1893	F6	-	2	Acy-CLEEWGASL-Tic-QCSG	9.0*10 ⁻⁶	-
S257	1894	F2	-	1	RWPNFYGYFESLLTHFS	1.4*10 ⁻⁵	0
S259	1895	F2	-	1	EGWDFYSYFSGLLASVT	7.7*10 ⁻⁶	0
S260	1896	F2	-	1	LDRQFYRYFQDLLVGFM	2.3*10 ⁻⁶	0
S261	1897	F2	-	1	WGRSFYRYFETLLAQGI	>2*10 ⁻⁵	0
S262	1898	F4	-	1	PLCFLOELFGGASLGGYCSG	1.9*10 ⁻⁵	0
S263	1899	F6	-	2	WLEQERAWWCEIQGGCRA	>2*10 ⁻⁵	0
S264	1900	F1	-	1	IQGWEPFYGWFDVVAQMFEE	1.9*10 ⁻⁷	0
S265	1901	F1	-	1	TGHRGLDEQFYWWFRDALSG	1.1*10 ⁻⁷	0
S266	1902	F6	-	2	Abu-CLEEWGASL-Tic-QCSG	>2*10 ⁻⁵	0
S268	1903	F1	-	1	RD-Hyp-FYDWFDDI	4.5*10 ⁻⁷	0
S273	1904	F1-F2	C-N	1-1	FHENFYDWFVRQVSKK-Lig-14-Lig-SDGFYNAIELLS	1.5*10 ⁻⁶	+
S278	1905	F1-derived	-	1	GFREGQRWYWFVAQVT	>2*10 ⁻⁵	0
S281	1906	F5	-		DLRVLCELFGGAYVLGYCSE	1.1*10 ⁻⁵	0

S282	1907	F4-derived	-			HL SVGEELSWWVALLGQWAR	>2*10 ⁻⁵	0
S283	1908	F4-derived	-			APVSTEELRWGALLFGQWAG	>2*10 ⁻⁵	0
S284	1909	F6-derived	-			ALEEEWAWVQVRSIRSGPL	>2*10 ⁻⁵	0
S285	1910	F6-derived	-			WLEHEWAQIQCELYGRGCTY	8.3*10 ⁻⁷	
S287	1911	F1	-	1		QAPSNFYDWFVREWDEE	5.9*10 ⁻⁶	0
S288	1912	F2	-	1		QSFYDIEELLGGEWKK	4.3*10 ⁻⁶	0
S289	1913	F2	-	1		DPFYQGLWEWLRESGEE	>2*10 ⁻⁵	0
S290	1914 and 1915	F1-F1	N-N	1-1		7-(Lig-GGGFHENFYDWFVRQVSKK) ₂	9.0*10 ⁻⁷	++
S291	1916 and 1917	F1-F1	N-N	1-1		9-(Lig-GGGFHENFYDWFVRQVSKK) ₂	1.2*10 ⁻⁶	+++
S292	1918 and 1919	F1-F1	N-N	1-1		12-(Lig-GGGFHENFYDWFVRQVSKK) ₂	7.5*10 ⁻⁷	++
S293	1920 and 1921	F1-F1	N-N	1-1		13-(Lig-GGGFHENFYDWFVRQVSKK) ₂	1.2*10 ⁻⁷	++
S294	1922	F1	-	1		DWLQRNANFYDWFVAEL-Lig	1.3*10 ⁻⁷	++
S295	1923	F1	-	1		Lig-DWLQRNANFYDWFVAEL	4.8*10 ⁻⁷	+
S300	1924 and 1925	F1-F1	C-C	1-1		(DWLQRNANFYDWFVAEL-Lig) ₂ -14	5.0*10 ⁻⁸	+++
S301	1926 and 1927	F1-F1	N-N	1-1		14-(Lig-DWLQRNANFYDWFVAEL) ₂	6.4*10 ⁻⁷	+
S302	1928	F2	-	1		SDGFYNA-Acy-ELLSG	8.6*10 ⁻⁷	0
S303	1929	F2	-	1		SGPFYEE-Acy-ELLW-Aib-G	5.7*10 ⁻⁶	0
S304	1930	F2	-	1		GGSFYDD-Acy-E-Aib-LW-Aib-G	2.1*10 ⁻⁵	0
S305	1931	F2	-	1		N-Aib-PFYDE-Acy-DE-Cha-W-Aib-G	8.4*10 ⁻⁷	0
S306	1932	F1	-	1		GRVDWLQRNANFYDWFVAE-Acy-G	2.2*10 ⁻⁶	+++
S312	1933 and 1934	F1-F1	N-N	1-1		23-(Lig-GGGFHENFYDWFVRQVSKK) ₂	2.9*10 ⁻⁶	++
S313	1935 and 1936	F2-F2	C-C	1-1		(SDGFYNAIELLS-Lig) ₂ -23	2.4*10 ⁻⁷	
S315	1937	F1	-	1		WFDWFWFE	6.8*10 ⁻⁶	0
S316	1938	F10	-	1		WQGYAWLS	7.0*10 ⁻⁶	0
S317	1939	F10	-	1		WPGYAWLS	>2*10 ⁻⁵	0
S319	1940	F1	-	1		D-Aic-D-Aib-EFYDWFDEIPq	8.7*10 ⁻⁷	0
S320	1941	F1	-	1		KNNKEFYWFDEIGq	2.8*10 ⁻⁶	0
S321	1942	F1	-	1		YeRD-Hyp-FYDWFDEIGq	1.4*10 ⁻⁶	0
S322	1943	F1	-	1		EWRD-Hyp-FYDWFDEI-Hyp-e	7.2*10 ⁻⁷	0
S325	1944 and 1945	F1-F1	N-N	1-1		9-(Lig-GSLDESFYDWFERQLGKK) ₂	4.6*10 ⁻⁸	++++

S326	1600	F1	-	1	1	GIQSQSPESFYDWFAGQVSDPWWCW	5.9*10 ⁻⁷	-
S327	1946	F2	-	1	1	TFYSCIASLLTGTQPNRGPWERCRRK	2.1*10 ⁻⁶	-
S329	1947 and 1948	F1-F1	N-N	1-1	1-1	17-(Lig ⁺ -FHENFYDWFVRQVSKK) ₂	2.7*10 ⁻⁶	++
S331	1949	F4	-	2	2	KHLCVLEELFWGASLFGYCSGKK	1.6*10 ⁻⁶	0
S332	1950 and 1951	F1-F1	C-C	1-1	1-1	(GSLDESFYDWFERQLGKK-Lig ⁺) ₂ -9	2.1*10 ⁻⁸	++++
S333	1952 and 1953	F1-F1	N-N	1-1	1-1	22-(Lig ⁺ -GSLDESFYDWFERQLGKK) ₂	1.4*10 ⁻⁷	++++
S334	1954 and 1955	F1-F1	N-N	1-1	1-1	22-(Lig ⁺ -GGGFHENFYDWFVRQVSKK) ₂	1.6*10 ⁻⁶	+++
S335	1956 and 1957	F1-F1	C-C	1-1	1-1	(GSLDESFYDWFERQLGKK-Lig ⁺) ₂ -22	9.8*10 ⁻⁸	++++
S336	1958 and 1959	F1-F1	N-N	1-1	1-1	23-(Lig ⁺ -GSLDESFYDWFERQLGKK) ₂	1.5*10 ⁻⁸	+++
S337	1960 and 1961	F1-F1	C-C	1-1	1-1	(GSLDESFYDWFERQLGKK-Lig ⁺) ₂ -23	1.1*10 ⁻⁸	++++
S342	1962	F1	-	1	1	DLWNAKEDMNFYDWFVWQLR	1.8*10 ⁻⁶	0
S344	1963	F2	-	1	1	EHWNTVDPFYHWISELLRESGA	2.0*10 ⁻⁷	0
S345	1964	F2	-	1	1	EHWNTVDPFYQYFAELLRESGA	2.9*10 ⁻⁶	0
S349	1965 and 1966	F1-F1	N-N	1-1	1-1	23-(Lig ⁺ -GGGFHENFYDWFVRQVSKK) ₂	1.3*10 ⁻⁷	+++
S350	1967 and 1968	F1-F1	C-C	1-1	1-1	(GSLDESFYDWFERQLGKK-Lig ⁺) ₂ -21	4.7*10 ⁻⁷	+++
S351	1969 and 1970	F1-F1	N-N	1-1	1-1	21-(Lig ⁺ -GSLDESFYDWFERQLGKK) ₂	1.4*10 ⁻⁶	+++
S352	1971 and 1972	F1-F1	N-N	1-1	1-1	21-(Lig ⁺ -GGGFHENFYDWFVRQVSKK) ₂	6.6*10 ⁻⁷	+++
S353	1973 and 1974	F1-F1	C-C	1-1	1-1	(GSLDESFYDWFERQLGKK-Lig ⁺) ₂ -14	1.1*10 ⁻⁸	++++
S354	1975 and 1976	F1-F1	N-N	1-1	1-1	14-(Lig ⁺ -GSLDESFYDWFERQLGKK) ₂	3.9*10 ⁻⁸	+++
S359	1977 and 1978	F1-F1	N-N	1-1	1-1	9-(Lig ⁺ -DWLORNANFYDWFVAEL) ₂	7.0*10 ⁻⁷	+
S360	1979 and 1980	F1-F1	N-N	1-1	1-1	23-(Lig ⁺ -DWLORNANFYDWFVAEL) ₂	9.9*10 ⁻⁷	
S361	1981 and 1982	F1-F1	C-C	1-1	1-1	(GSLDESFYDWFERQLGKK-Lig ⁺) ₂ -24	2.2*10 ⁻⁶	+++
S362	1983 and 1984	F1-F1	N-N	1-1	1-1	24-(Lig ⁺ -GSLDESFYDWFERQLGKK) ₂	1.1*10 ⁻⁷	+++
S363	1985 and 1986	F1-F1	N-N	1-1	1-1	24-(Lig ⁺ -GGGFHENFYDWFVRQVSKK) ₂	2.2*10 ⁻⁷	+++
S365	1987	F1	-	1	1	RMVFSTGAPQNFYDWFVQEW	1.0*10 ⁻⁵	0
S366	1988	F1	-	1	1	PLRESNFYDWFVQQL	3.7*10 ⁻⁷	0
S368	1989	F2	-	1	1	RGTRSDPFYHKLSELLQGH	>2*10 ⁻⁵	0
S371	1558	F1	-	1	1	GSLDESFYDWFERQLGKK	6.3*10 ⁻⁷	+
S372	1990	F1	-	1	1	SGSLDESFYDWFERQLGKK	2.0*10 ⁻⁷	++
S373	1991	F1	-	1	1	GSLDESFYDWFERQLGKK(S)	1.2*10 ⁻⁷	+++
S374	1992 and 1993	F1-F1	N-N	1-1	1-1	17-(Aid-GSLDESFYDWFERQLGKK) ₂	1.8*10 ⁻⁷	+++
S375	1994	F1-F1	C-N	1-1	1-1	(GSLDESFYDWFERQLGKK-Aid)-14-(Aid-GSLDESFYDWFERQLGKK)	2.0*10 ⁻⁷	+++

S376	1995 and 1996	F1-F1	N-N	1-1	19-(Aid-GSLDESYDWFERQLGKK) ₂	1.6*10 ⁻⁷	++++
S378	1997 and 1998	F1-F1	C-C	1-1	(GSLDESYDWFERQLGKKK-Aid) ₂₋₁₇	6.5*10 ⁻⁸	+++++
S379	1999 and 2000	F1-F1	C-C	1-1	(GSLDESYDWFERQLGKKK-Aid) ₂₋₁₉	5.6*10 ⁻⁸	+++++
S380	2001 and 2002	F1-F1	C-C	1-1	(EEDWLQRNANFYDWFWAEL-Lig) ₂₋₉	5.1*10 ⁻⁷	++
S381	2003 and 2004	F1-F1	C-C	1-1	(EEDWLQRNANFYDWFWAEL-Lig) ₂₋₂₃	1.2*10 ⁻⁷	++++
S386	1559	F1	-	1	GSLDESYDWFERQLG	3.2*10 ⁻⁷	+
S387	2005	F1	-	1	SLDESYDWFERQLG	6.3*10 ⁻⁷	+
S388	2006	F1	-	1	LDESYDWFERQLG	3.4*10 ⁻⁷	+
S389	2007	F1	-	1	DESYDWFERQLG	1.1*10 ⁻⁶	+
S390	1794	F1	-	1	ESFYDWFERQLG	6.2*10 ⁻⁷	+
S391	2008	F1	-	1	SYDWFERQLG	1.5*10 ⁻⁶	+
S392	2009	F1	-	1	FYDWFERQLG	3.8*10 ⁻⁶	0
S394	1788	F1	-	1	GSLDESYDWFERQ	9.1*10 ⁻⁸	+
S395	1787	F1	-	1	GSLDESYDWFERQL	8.1*10 ⁻⁸	++
S396	1789	F1	-	1	GSLDESYDWFER	>2*10 ⁻⁵	0
S397	2010	F1	-	1	GSLDESYDWFE	>2*10 ⁻⁵	0
S398	2011	F1	-	1	GSLDESYDWF	>2*10 ⁻⁵	0
S399	1790	F1	-	1	ESFYDWFERQL	9.5*10 ⁻⁸	++
S400	1791	F1	-	1	ESFYDWFERQ	6.3*10 ⁻⁷	0
S401	2012	F1	-	1	ESFYDWFER	>2*10 ⁻⁵	0
S402	2013	F1	-	1	ESFYDWFE	>2*10 ⁻⁵	0
S403	2014	F1	-	1	ESFYDW	>2*10 ⁻⁵	0
S414	2015 and 2016	F1-F1	C-C	1-1	(ESFYDWFERQLGK-Lig) ₂₋₁₄	3.8*10 ⁻⁷	++++
S415	2017 and 2018	F1-F1	C-C	1-1	(ESFYDWFERQLGK-Lig) ₂₋₂₃	1.0*10 ⁻⁷	++++
S416	2019 and 2020	F1-F1	N-N	1-1	14-(Lig-ESFYDWFERQLG) ₂	9.3*10 ⁻⁷	+++
S417	2021 and 2022	F1-F1	N-N	1-1	23-(Lig-ESFYDWFERQLG) ₂	9.2*10 ⁻⁷	+++
S418	2023 and 2024	F1-F1	C-C	1-1	(ESFYDWFERQLGK-Aid) ₂₋₁₇	1.2*10 ⁻⁷	++++
S419	2025 and 2026	F6-F6	N-N	2-2	14-(Lig-EWLDQEWAWVQCEVYGRGCPSEE) ₂		0
S420	2027 and 2028	F1-F1	N-N	1-1	17-(Aid-ESFYDWFERQLG) ₂		++
S423	2029 and 2030	F1-F8	C-C	1-3	ESFYDWFERQLG K ACAWPTYWNCG	6.2*10 ⁻⁸	0

S425	2031	F1-F6	C-N	1-2	GSLDESFDWFERQLGKK-Lig'-14- Lig'-EWLDQEWAWVQCEVYGRGCPSEE	2.4*10 ⁻⁹	-
S429	2032	F6-F1	C-N	2-1	EWLDQEWAWVQCEVYGRGCPSEE-Lig' -14-Lig'-GSLDESFDWFERQLGKK	6.0*10 ⁻¹⁰	
S432	2033 and 2034	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K CEVYGRGCP	1.8*10 ⁻⁷	+
S433	2035 and 2036	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K WLDQEWAWVQ	1.1*10 ⁻⁷	+
S436	2037 and 2038	F1-F6	C-C	1-2	ESFYDWFERQLGGGG K WLDQEWAWVQCEVYGRGCP	5.2*10 ⁻¹⁰	+++
S437	2039 and 2040	F1-F6	C-C	1-2	WLDQEWAWVQCEVYGRGCP ESFYDWFERQLGGGG K	6.9*10 ⁻¹⁰	+++
S438	2041 and 2042	F1-F6	C-C	1-2	LDQEWAWVQCEVYGRGCP ESFYDWFERQLGGGG K	3.0*10 ⁻⁸	++
S439	2043 and 2044	F1-F6	C-C	1-2	DQEWAWVQCEVYGRGCP ESFYDWFERQLGGGG K	4.6*10 ⁻⁸	
S440	2045 and 2046	F1-F6	C-C	1-2	QEWAWVQCEVYGRGCP ESFYDWFERQLGGGG K	9.9*10 ⁻⁸	
S441	2047 and 2048	F1-F6	C-C	1-2	EWAWVQCEVYGRGCP ESFYDWFERQLGGGG K	1.2*10 ⁻⁷	
S442	2049 and 2050	F1-F6	C-C	1-2	WAWVQCEVYGRGCP ESFYDWFERQLGGGG K	1.6*10 ⁻⁷	
S443	2051 and 2052	F1-F6	C-C	1-2	AWVQCEVYGRGCP ESFYDWFERQLGGGG K	1.7*10 ⁻⁷	
S444	2053 and 2054	F1-F6	C-C	1-2	WVQCEVYGRGCP ESFYDWFERQLGGGG	1.9*10 ⁻⁷	

S445	2055 and 2056	F1-F6	C-C			VOCEVYGRGCP K ESFYDWFERQLGGG K QCEVYGRGCP		2.3*10 ⁻⁷	
S453	2057	F1-F6	C-N	1-2		GSLDESFYDWFERQLGKK-Aid-17-Aid-KEWLDQEWAWVQCEVYGRGCPSEE		5.7*10 ⁻¹⁰	-
S454	2058 and 2059	F1-F6	C-C	1-2		GSLDESFYDWFERQLGKK-Aid-17		3.8*10 ⁻¹⁰	+++
S455	2060	F6-F1	C-N	2-1		EWLDQEWAWVQCEVYGRGCPSEEK-Aid		1.1*10 ⁻⁹	++++
S456	2061 and 2062	F1-F6	N-N	1-2		EWLDQEWAWVQCEVYGRGCPSEEK-Aid-18-Aid-GSLDESFYDWFERQLGKK 17 Aid-GSLDESFYDWFERQLGKK		2.4*10 ⁻⁹	+++
S457	2063	F6-F1	C-N	2-1		Aid-KEWLDQEWAWVQCEVYGRGCPSEE		1.6*10 ⁻⁹	++++
S458	2064	F6-F1	C-N	2-1		WLDQEWAWVQCEVYGRGCPGGSGSLDESFYDWFERQLG		3.2*10 ⁻⁹	++++
S459	2065	F1-F6	C-N	1-2		WLDQEWAWVQCEVYGRGCPGGSGSLDESFYDWFERQLG		7.6*10 ⁻¹¹	-
S467	2066	F6-F1	C-N	2-1		GSLDESFYDWFERQLGKK-Aid-16-Aid-GSLDESFYDWFERQLGKK		6.8*10 ⁻¹⁰	++++
S468	2067	F6-F1	C-N	2-1		EWLDQEWAWVQCEVYGRGCPSEEK-Aid-19-Aid-GSLDESFYDWFERQLGKK		4.0*10 ⁻¹⁰	++++
S471	2068	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG		6.7*10 ⁻¹⁰	++++
S481	2069	F6-F1	C-N	2-1		HHHHHKLQDEWAWVQCEVYGRGCPSESYDWFERQLG			
S482	2070	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG			
S483	2071	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG		5.2*10 ⁻⁸	0
S484	2072	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG		8.7*10 ⁻⁸	0
S485	2073	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG		1.6*10 ⁻⁷	0
S486	2074	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG		5.7*10 ⁻⁸	0
S487	2075	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG			
S488	2076	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG			
S489	2077	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG			
S490	2078	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG			
S491	2079	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG			
S492	2080	F6-F1	C-N	2-1		LDQEWAWVQCEVYGRGCPSESYDWFERQLG			

S493	2081	F6-F1	C-N	2-1	EWLQEWAWWQCEVYGRGCPSEE-POX-Lys(biotin)		
S494	2082	F6-F1	C-N	2-1	ADQEWAWWQCEVYGRGCPSESYDWFERQLG	1.7*10 ⁻⁸	+
S495	2083	F6-F1	C-N	2-1	LAQEWAWWQCEVYGRGCPSESYDWFERQL		
S496	2084	F6-F1	C-N	2-1	LDAEWAWWQCEVYGRGCPSESYDWFERQL		
S497	2085	F6-F1	C-N	2-1	LDQAWAWWQCEVYGRGCPSESYDWFERQL	2.5*10 ⁻⁹	+++
S498	2086	F6-F1	C-N	2-1	LDQAAWVQCEVYGRGCPSESYDWFERQL	5.6*10 ⁻⁸	+
S499	2087	F6-F1	C-N	2-1	LDQEWAAVQCEVYGRGCPSESYDWFERQL	6.2*10 ⁻¹⁰	+++
S500	2088	F6-F1	C-N	2-1	LDQEWAWQCEVYGRGCPSESYDWFERQL		
S501	2089	F6-F1	C-N	2-1	LDQEWAWVACEVYGRGCPSESYDWFERQL		
S502	2090	F6-F1	C-N	2-1	LDQEWAWVQCAVYGRGCPSESYDWFERQL	3.0*10 ⁻⁹	+++
S503	2091	F6-F1	C-N	2-1	LDQEWAWVQCAVYGRGCPSESYDWFERQL		
S504	2092	F6-F1	C-N	2-1	LDQEWAWVQCAVYGRGCPSESYDWFERQL		
S505	2093	F6-F1	C-N	2-1	LDQEWAWVQCAVYGRGCPSESYDWFERQL		
S506	2094	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESYDWFERQL		
S507	2095	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESYDWFERQL		
S508	2096	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESYDWFERQL		
S509	2097	F6-F1	C-N	2-1	LDQEWAWVQCEVYGRGCPSESYDWFERQL		
S510	2098	F6-F1	C-N	2-1	SLEEEWAQVECEVYGRGCPSSGSGGLDESFYHWFDRQLR	6.2*10 ⁻¹¹	++++
S511	2099	F6-F1	C-N	2-1	WLDQEWAWWQCEVYGRGCPSSGSGGRVDWLQRNANFYDWFVAELG	3.8*10 ⁻⁹	++
S512	2100	F6-F1	C-N	2-1	WLDQEWAWWQCEVYGRGCPSSGSGGSQAGSAFYAWFDQVLRV	2.8*10 ⁻⁸	++
S513	2101	F6-F1	C-N	2-1	WLDQEWAWWQCEVYGRGCPSSGSGGSQDAFYSGLWALIGLSDG		
S515	2102	F6	-	2	LDQEWAWWQCEVYGRGCPSPOX-Lys(Biotin)		
S516	2103	F4-F1	C-N	2-1	H-Acy-CLEEWGASL-Tic-QCSGSESYDWFERQL		
S517	2104	F6-F1	C-N	2-1	SIEEEWAQIKDWWGRGCPSESYDWFERQL		
S518	2105	F6-F1	C-N	2-1	RLEEEWAWWQCEVYGRGCPSSGSLDESFYDWFERQLG	1.6*10 ⁻¹⁰	++++
S519	2106	F6-F1	C-N	2-1	SLEEEWAQVECEVYGRGCPSSGSLDESFYDWFERQLG	2.0*10 ⁻¹¹	+++++
S520	2107	F6-F1	C-N	2-1	SIEEEWAQIKDWWGRGCPPLLDDESFYHWFDRQLR	2.0*10 ⁻¹¹	+++++
S521	2108	F4-F1	C-N	2-1	HLCVLEELFWGASLFGYCSGGSLDESFYDWFERQL	2.7*10 ⁻⁸	+
S522	2109	F4-F1	C-N	2-1	HLCVLEELFWGASLFGYCSGGGRVDWLQRNANFYDWFVAELG		
S523	2110	F6-F10	C-N	2-1	WLDQEWAWWQCEVYGRGCPSSDSDWAGYEWFEQLD	4.3*10 ⁻⁹	++
S524	2111	F6-F1	C-N	2-1	HHHHKSLLEEWAQVECEVYGRGCPSSGSLDESFYDWFERQLG		

7, 9, 12, 13, 14, 17, 19, 20, 21, 22, 23, and 24 represent specific chemical linkers (see Table 3); For FFC: 0 is no effect, + is agonist, - is antagonist. Peptides listed on 3 lines consist of two different peptides, linked N-N or C-C, either by chemical linkage or by being synthesized on the two branches of an amino acid with two amino groups such as, e.g., lysine. Acy = 1-amino-1-cyclohexanecarboxylic acid; Cha = cyclohexylalanine; Alb = 2-aminoisobutyric acid; Hyp = Hydroxyproline; Amino acids which are not capitalized are D-amino acids; Lig = Diaminopropionic acid with a 2-aminohydroxyacetyl group (CO-CH₂-O-NH₂) on the side chain amino group; Lig' = lysine with a 2-aminohydroxyacetyl group (CO-CH₂-O-NH₂) on the side chain amino group; Ald = an aldehyde group obtained by periodate oxidation of a serine, either N-terminal or attached to the side chain amino group of lysine.

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Results further indicated that S175-S175 dimer peptides (Site 1-Site 1) were less agonistic than S175 monomer peptides (++ vs. +++). S175-S175 dimer peptides having a C-N linkage were less agonistic or equally agonistic as compared to S175-S175 dimer peptides having C-C or N-N linkages. F8-F8 dimer peptides, like the parent monomer, showed no agonist activity.

Example 5: Substrate Phosphorylation Assay (HIR Kinase)

WGA (wheat germ agglutinin)-purified recombinant human insulin receptor was mixed with either insulin or peptide in varying concentrations in substrate phosphorylation buffer (50 mM HEPES (pH 8.0), 3 mM MnCl_2 , 10 mM MgCl_2 , 0.05% Triton X-100, 0.1% BSA, 12.5 μM ATP). A synthetic biotinylated substrate peptide (Biotin-KSRGDYMTMQIG) was added to a final concentration of 2 $\mu\text{g/ml}$. Following a 1 h incubation at RT, the reactions were stopped by the addition of 50 mM EDTA. The reactions were transferred to Streptavidin coated 96-well microtiter plates (NUNC, Cat. No. 236001) and incubated for 1 h at RT. The plates were washed 3 times with TBS (10 mM Tris (pH 8.0), 150 mM NaCl).

Subsequently, a 2000-fold dilution of horseradish peroxidase (HRPO) conjugated phosphotyrosine antibody (Transduction Laboratories, Cat. No. E120H) in TBS was added. The plates were incubated for 30 min and washed 3 times with TBS. TMB (3,3',5,5'-tetramethylbenzidine; Kem-En-Tec, Copenhagen, Denmark) was added. One substrate from Kem-En-Tec was added. After 10-15 min, the reaction was stopped by the addition of 1% acetic acid. The absorbance, representing the extent of substrate phosphorylation, was measured in a spectrophotometer at a wavelength of 450 nM.

The results indicated that the potency of the Site 1-Site 2 dimer, peptide 539, was 0.1 to 1% of that of insulin in all assays tested (Table 8), and the dose-response curves (Figures 17A-17B) had a shape similar to that of insulin dose-response curves, suggesting an insulin-like action

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mechanism. In addition, Site 1-Site 2 dimer peptides 537 and 538 were also active as specific insulin receptor antagonists (Table 8; Figures 16A-16C). Notably, Site 2-Site 1 dimer peptide 539 was more active in the kinase assay than Site 1-Site 1 homodimer peptides 521 and 535 (Figures 19A-19B), despite lower FFC potency (Figures 14A-14C; Figures 17A-17B). Similar results are shown in Figures 20A-B and Figures 21A-B. This data suggested that homodimer and heterodimer peptides used different mechanisms of action.

TABLE 8

Pep.	Mon./Link.	Sequence	SEQ ID NO:	Form	Site IR	HIR K _d (nM)	HIGF-1R K _d (nM)	FFC Pot. (nM)	Kinase Pot. (nM)
HI				na	na				
HIGF-1R				na	na				
521	RP9-6aa-RP9	MADYKDDDDKGS LD ESFYDWFE RQLGKKGGSGGSGSLDESFYDW FERQLGKKAAA(ETAG)PG	2112	1-1	1-1	25	-	A 3	1400
535	RP9-12aa-RP9	MADYKDDDDKGS LD ESFYDWFE RQLGKKGGSGGSGGSGGSGSL DESFYDWFERQLGKKAAA(ETAG) JPG	2113	1-1	1-1	15	-	A 2	1000
537	RP9-6aa-D8	MADYKDDDDKGS LD ESFYDWFE RQLGKKGGSGGSGWLDQEWAWV QCEVYGRGCP S AAA(ETAG)PG	2114	1-6	1-2	0.092	980	N 10	Inactive
538	RP9-12aa-D8	MADYKDDDDKGS LD ESFYDWFE RQLGKKGGSGGSGGSGGSGWLD QEWAWVQCEVYGRGCP S AAA(E TAG)PG	2115	1-6	1-2	0.080	710	N 10	Inactive
539	D8-6aa-RP9	MADYKDDDDKWLDQEWAWVQC EVYGRGCPSGGSGGSGSLDES FYDWFERQLGKKAAA(ETAG)PG	2116	6-1	2-1	0.530	1500	A 10	110

A = agonist; N = antagonist; na = not applicable; Form. = formula; Mon. = constituent monomers; Link. = linker; Pot. = potency; HI and HIGF-1R are controls; All with tags at both ends; All dimers are linked C-N; Linker sequences are underlined.

15 Example 6: IR Autophosphorylation Assays

IR activation was assayed by detecting autophosphorylation of an insulin receptor construct transfected into 32D cells (Wang *et al.*, 1993, *Science* **261**:1591-1594; clone 969). The IR transfected 32D cells were

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seeded at 5×10^6 cells/well in 96-well tissue culture plates and incubated overnight at 37°C. Samples were diluted 1:10 in the stimulation medium (PRIM1640 with 25 nM HEPES pH 7.2) plus or minus insulin. The culture media was decanted from the cell culture plates, and the diluted samples
5 were added to the cells. The plates were incubated at 37°C for 30 min. The stimulation medium was decanted from the plates, and cell lysis buffer (50 mM HEPES pH 7.2, 150 mM NaCl, 0.5% Triton X-100, 1 mM AEBSF, 10 KIU/ml aprotinin, 50 µM leupeptin, and 2 mM sodium orthovanadate) was added. The cells were lysed for 30 min.

10 In the ELISA portion of the assay, the cell lysates were added to the BSA-blocked anti-IR unit mAb (Upstate Biotechnology, Lake Placid, NY) coated ELISA plates. After a 2 h incubation, the plates were washed 6 times with PBST and biotinylated anti-phosphotyrosine antibody (Upstate Biotechnology) is added. After another 2 h incubation, the plates were
15 again washed 6 times. Streptavidin-Eu was then added, and the plates were incubated for 1 h. After washing the plates again, EG&G Wallac enhancement solution (100 mM acetone-potassium hydrogen phthalate, pH 3.2; 15 mM 2-naphthyltrifluoroacetate; 50 mM tri(n-octyl)-phosphine oxide; 0.1% Triton X-100) was added into each well, and the plates were placed
20 onto a shaker for 20 min at RT. Fluorescence of samples in each well was measured at 615 nm using a VICTOR 1420 Multilabel Counter (EG&G Wallac).

Alternatively, IR autophosphorylation was determined using a holoenzyme phosphorylation assay. In accordance with this assay, 1 µl of
25 purified insulin receptor (isolated from a Wheat Germ Agglutinin Expression System) was incubated with 25 nM insulin, or 10 or 50 µM peptide in 50 µl autophosphorylation buffer (50 mM HEPES pH. 8.0, 150 mM NaCl, 0.025% Triton-X-100, 5 mM MnCl₂, 50 µM sodium orthovanadate) containing 10 µM ATP for 45 min at 22°C. The reaction was stopped by adding 50 µl of gel
30 loading buffer containing β-mercaptoethanol (Bio-Rad Laboratories, Inc., Hercules, CA). The samples were run on 4-12% SDS-polyacrylamide gels.

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Western Blot analysis was performed by transferring the proteins onto nitrocellulose membrane. The membrane was blocked in PBS containing 3% milk overnight. The membrane was incubated with anti-phosphotyrosine 4G10 HRP labeled antibody (Upstate Biotechnology) for 2 h. Protein bands
5 were visualized using SuperSignal West Dura Extended Duration Substrate Chemiluminescence Detection System (Pierce Chemical Co.).

Example 7: Fluorescence-Based HIR Binding Assays

A. Time-Resolved Fluorescence Resonance Energy Transfer Assays

10 Time-resolved fluorescence resonance energy transfer assays (TR-FRET) were used for peptide competition studies. In one set of assays, monomer and dimer peptides were tested for the ability to compete with biotinylated RP9 monomer peptide (b-RP9) for binding to HIR-immunoglobulin heavy chain chimera (sIR-Fc; Bass *et al.*, 1996). The
15 assays were performed using a 384-well white microplate (NUNC) with a final volume of 30 μ l. Final incubation conditions were in 22 nM b-RP9, 1 nM SA-APC (streptavidin-allophycocyanin), 1 nM Eu^{3+} -sIR-Fc (LANCE™ labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA (Cohn Fraction V). After 16-24 h of incubation
20 at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor² 1420 plate reader (PE Wallac, Inc.). Primary data were background corrected, normalized to buffer controls, and then expressed as percent of specific binding.

Results are shown in Figures 22A-22B. Figure 21A shows b-RP9
25 competition data. For these figures, the Z'-factor was greater than 0.5 ($Z' = 1 - (3\sigma_+ + 3\sigma_-) / |\mu_+ - \mu_-|$; Zhang *et al.*, 1999, *J. Biomol. Screen.* 4:67-73), and the signal-to-background (S/B) ratio was ~4-5. In Figure 22A, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data according

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to the following formula: $y = \min + (\max - \min) / (1 + 10^{((\log IC_{50} - x) * \text{Hillslope}))}$. This was used to determine IC_{50} values.

In another set of assays, monomer and dimer peptides were tested for the ability to compete with biotinylated-S175 (b-S175) or b-RP9 for binding to sIR-Fc. The TR-FRET assays were performed in a 384-well white microplate with a final volume of 30 μ l. Final incubation conditions were in 33 nM b-S175 or 22 nM b-RP9, 1 nM SA-APC, 1 nM Eu^{3+} -sIR-Fc, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA. After 16-24 h of incubation at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor² 1420 plate reader. Primary data were background corrected, normalized to buffer controls, and then expressed as % specific binding.

Results are shown in Figures 23A-23B. For these figures, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data, which was used to determine IC_{50} values. Figure 23A shows b-S175 competition data; Figure 23B shows b-RP9 competition data.

B. Fluorescence Polarization Assays

Fluorescence polarization assays (FP) were used for peptide competition studies. In one set of assays monomer and dimer peptides were tested for the ability to compete with fluorescein-RP9 (FITC-RP9) for binding to soluble HIR ectodomain (sIR; Kristensen *et al.*, 1998, *J. Biol. Chem.* **273**:17780-17786). The assays were performed in a 384-well black microplate (NUNC) with a final volume of 30 μ l. Final incubation conditions were 1 nM FITC-RP9, 10 nM sIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG (bovine gamma globulin), 0.005% Tween-20[®]. After 16-24 h of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst[™] AD plate reader (LJL BioSystems, Inc.). Primary data were background corrected using 10 nM sIR without FITC-RP9

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addition, normalized to buffer controls, and then expressed as percent of specific binding. The Z'-factor was greater than 0.5 and the assay dynamic range was ~125 mP. In Figures 24-27, each data point represents the average of two replicate wells. The lines represent the best fit to a four-
5 parameter non-linear regression analysis of the data, which was used to determine IC₅₀ values. The Z'-factor was greater than 0.5 and the assay dynamic range was ~125 mP. Results are shown in Figures 24A-24B.

In another set of assays, monomer and dimer peptides were tested for the ability to compete with FITC-RP9 for binding to soluble human insulin
10 mini-receptor (mlR; Kristensen *et al.*, 1999, *J. Biol. Chem.* **274**:37351-37356). The FP assays were performed in a 384-well black microplate with a final volume of 30 μ l. Final incubation conditions were 2 nM FITC-RP9, 20 nM mlR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.001% BGG, 0.005% Tween-20®. After 16-24 h of incubation at RT, the
15 fluorescence signal at 520 nm was read on an Analyst™ AD plate reader. Primary data were background corrected using 20 nM mlR without FITC-RP9 addition, normalized to buffer controls and then expressed as percent of specific binding. Results are shown in Figures 25A-25B.

Monomers and dimer peptides were also tested for the ability to
20 compete with fluorescein-insulin (FITC-Insulin) for binding to slR. The FP assays were performed in a 384-well black microplate with a final volume of 30 μ l. Final incubation conditions were in 2 nM FITC-Insulin, 20 nM slR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG, 0.005% Tween-20®. After 16-24 h of incubation at RT, the fluorescence
25 signal at 520 nm was read on an Analyst™ AD plate reader. Primary data were background corrected using 20 nM slR without FITC-Insulin addition, normalized to buffer controls and then expressed as percent of specific binding. Results are shown in Figures 26A-26B.

In other assays, peptide monomers and dimer peptides were tested
30 for the ability to compete with FITC-Insulin for binding to mlR. The FP

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assays were performed in a 384-well black microplate with a final volume of 30 μ l. Final incubation conditions were 2 nM FITC-Insulin, 20 nM mIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG (bovine gamma globulin), 0.005% Tween-20®. After 16-24 h of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst™ AD plate reader. Primary data were background corrected using 20 nM mIR without FITC-RP9 addition, normalized to buffer controls and then expressed as % specific binding. Results are shown in Figures 27A-27B.

C. Summary

Table 9, below, summarizes the binding data calculated from competition assays using the IR constructs, sIR-Fc, sIR, and mIR, in TR-FRET and FP formats. The data in Table 9 indicate that most dimer peptides (e.g., S291 and S375 or S337), showed greater agonist activity than the corresponding monomer peptides (e.g., H2C or RP9, respectively) in the FFC assay. It was previously demonstrated that an inequality between monomer peptides and insulin was exhibited in competition assays where the assay reporter was a monomer peptide (i.e., RP9 or S175). This inequality was also demonstrated by dimer peptides as seen in Table 9. Table 9 further shows that Group 6 monomer peptides such as E8 (D120) were able to compete with FITC-RP9 or b-RP9 peptides for binding to sIR-Fc, but did not compete peptide ligands, such as FITC-RP9 for binding to mIR. Experiments using different IR constructs thereby allowed differentiation of Site I peptides based on sequence motifs (i.e., Group 6 (Formula 10) vs. Group 1 (Formula 1; A6)).

TABLE 9

Monomer or Dimer	SEQ ID NO:	Linkage	TARGET ⇒		sIR-Fc		sIR-Fc		sIR-Fc		sIR		mIR		HIR	
			Label	⇒	b-S175		b-RP9		FITC-RP9		FITC-RP9		FITC-RP9		125I-insulin	
					FRET	Hill	IC50 (nM)	Hill	IC50 (nM)	Hill	FRET	FP	IC50 (nM)	Hill	RRA	FFC
H2C	2117		FHENFYDWFQVRVSKK		410	-0.82	1626	-1.03	50	-0.27	37	-0.49	770	-0.89	700	+
S291 and 1917	1916	N-N	(Lig-GGG-H2C) ₂ -9		81	-0.96	250	-0.69			12	-0.35	668	-0.38	1200	++++
RP9	1558		GSLDESFYDWFERQLGKK		6	-0.45	42	-0.69	10	-0.41	0.03	-0.29	49	-0.53	44	+0
S375	1994	C-N	(RP9-Lig)-14-(RP9-Lig)		7	-0.80	86	-0.67			0.2	-0.22	91	-0.80	200	++++
S337 and 1961	1960	C-C	(RP9-Lig) ₂ -23		0.2	-0.36	14	-0.57	1	-0.37	0.2	-0.28	111	-0.70	11	++++
S391	2008		truncated(-GSLDE)RP9(-KK)		59	-0.59	610	-0.56			119	-0.49	284	-0.77	1500	NN
S390	1794		truncated(-GSLD)RP9(-KK)		27	-0.49	127	-0.49			19	-0.64	94	-0.94	620	+
S414 and 2016	2015	C-C	(truncated(-GSLD)RP9(-KK)) ₂ -14		92	-0.62	164	-0.73			0.2	-0.25	151	-0.69	NN	NN
S175	1560		GRVDWLQRNANFYDWFVAELG		22	-0.58	64	-0.74	10	-0.56	1	-0.36	167	-1.72	230	+++
S380 and 2002	2001	C-C	(EE-short-S175-Lig) ₂ -9		10	-0.55	23	-0.64			0.5	-0.29	27	-0.49	510	++
E8 (D120)	2118		GGTWPGYEWLRNA		755	-0.74			207	-0.49			>100000		2200	-
Insulin					59	-0.37	63	-0.46	>100000	-0.25	1250	-	172	-0.78	0.04	++++

FRET = Time-Resolved Fluorescence Resonance Energy Transfer Assay; FP = Fluorescence Polarization Assay; RRA = Radio-Receptor Assay; FFC = Free Fat Cell Assay; N-N = N-terminal linkage; C-C = C-terminal linkage; All are site 1 (formula 1) monomers or site 1-site 1 (formula 1-formula 1) dimers;

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Based on the functional studies outlined above, the following peptide dimers were designed.

SEQ ID NO:	Monom./ Linkers	Sequence
2119	F8-6aa-RP9	HLCVLEELFWGASLFGYCSGGSGGSGSLDESFYDWFERQL
2120	F8-12aa-RP9	HLCVLEELFWGASLFGYCSGGSGGSGGSGGSGSLDESFYDWFERQL
2121	D8-6aa-S175	WLDQEWAWVQCEVYGRGCPSSGGSGGSGRVDWLQRNANFYDWFVAELG
2122	D8-12aa-S175	WLDQEWAWVQCEVYGRGCPSSGGSGGSGGSGGSGRVDWLQRNANFYDWFVAELG
2123	F8-6aa-S175	HLCVLEELFWGASLFGYCSGGSGGSGRVDWLQRNANFYDWFVAELG
2124	F8-12aa-S175	HLCVLEELFWGASLFGYCSGGSGGSGGSGGSGRVDWLQRNANFYDWFVAELG
2125	D8-6aa-RP15	HLCVLEELFWGASLFGYCSGGSGGSSQAGSAFYAWFDQVLRTV
2126	D8-6aa-RP6	HLCVLEELFWGASLFGYCSGGSGGSTFYSCLASLLTGTPQPNRGPWERCRCR
2127	D8-6aa-RP17	HLCVLEELFWGASLFGYCSGGSGGSSQSDAFYSGLWALIGLSDG
2128	D8-6aa-Grp 6	HLCVLEELFWGASLFGYCSGGSGGSSDSDWAGYEWFEELQD

- 5 Linker sequences are underlined and in bold; Monomer sequences are shown below; All dimers are linked C-N.

SEQ ID NO:	Monomer	Formula	Site	Sequence
1576	F8	4	2	HLCVLEELFWGASLFGYCSG
1558	RP9	1	1	GSLDESFYDWFERQL
2129	D8	6	2	WLDQEWAWVQCEVYGRGCP
1560	S175	1	1	GRVDWLQRNANFYDWFVAELG
2130	RP15	1	1	SQAGSAFYAWFDQVLRTV
1635	Rp6	2	1	TFYSCLASLLTGTPQPNRGPWERCRCR
2131	RP17	1	1	QSDAFYSGLWALIGLSDG
1595	Group 6	10	1	DSDWAGYEWFEELQD

Example 8: Peptide Fusions To The Maltose Binding Protein

10

A. Cloning

The transfer of interesting peptide sequences from phage display to maltose binding protein (MBP) fusions is desirable for several reasons. First, to obtain a more sensitive affinity estimate, the polyvalency of phage

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display peptides should be converted to a monovalent system. For this purpose, the peptide sequences are fused to MBP that generally exists as a monomer with no cysteine residues. Second, competition experiments can be carried out with the same or different peptides, one phage displayed and the other fused to MBP. Lastly, purified peptides can be obtained by cleavage of the fusion protein at a site engineered in the DNA sequence.

Figure 28 shows a schematic drawing of the MBP-peptide construct. In the construct, the N-terminus of the peptide sequence is fused to the C-terminus of the MBP. Two peptide-flanking epitope tags are included, a shortened-FLAG® at the N-terminus and E-Tag at the C-terminus. The corresponding gene fusion was generated by ligating a vector fragment encoding the MBP in frame with a PCR product encoding the peptide of interest. The vector fragment was obtained by digesting the plasmid pMAL-c2 (New England Biolabs) with *EcoRI* and *HindIII* and then treating the fragment with shrimp alkaline phosphatase (SAP; Amersham). The digested DNA fragment was resolved on a 1% agarose gel, excised, and purified by QIAEXII (QIAGEN). The 20-amino acid peptide sequences of interest were initially encoded in the phage display vector pCANTAB5E (Pharmacia). To obtain these sequences, primers were synthesized which anneal to sequences encoding the shortened FLAG® or E-Tag epitopes and also contain the required restriction enzyme sites *EcoRI* and *HindIII*. PCR products were obtained from individual phage clones and digested with restriction enzymes to yield the insert fragment. The vector and insert were ligated overnight at 15°C. The ligation product was purified using QIAquick spin columns (QIAGEN) and electroporations were performed at 1500 v in an electroporation cuvette (0.1 mm gap; 0.5 ml volume) containing 10 ng of DNA and 40 µl of *E. coli* strain ER2508 (RR1 *lon::miniTn10*(Tet^r) (*malB*) (*argF-lac*)U169 Pro⁺ *zjc::Tn5*(Kan^r) *fhuA2*) electrocompetent cells (New England Biolabs). Immediately after the pulse, 1 ml of pre-warmed (40°C) 2xYT medium containing 2% glucose (2xYT-G) was added and the transformants were grown at 37°C for 1 h. Cell transformants were plated

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onto 2xYT-AG plates and grown overnight at 37°C. Sequencing confirmed the clones contained the correct constructs.

B. Small-Scale Expression of Soluble MBP-Peptide Fusion Proteins

5 *E. coli* ER2508 (New England Biolabs) carrying the plasmids encoding MBP-peptide fusion proteins were grown in 2xYT-AG at 37°C overnight (250 rpm). The following day the cultures were used to inoculate media (2x YT containing-G) to achieve an OD₆₀₀ of 0.1. When the cultures reached an OD₆₀₀ of 0.6, expression was induced by the addition of IPTG to
10 a final concentration of 0.3 mM and then cells were grown for 3 h. The cells were pelleted by centrifugation and samples from total cells were analyzed by SDS-PAGE electrophoresis. The production of the correct molecular weight fusion proteins was confirmed by Western blot analysis using the monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia).

15 **C. Large-Scale Expression of Soluble MBP-Peptide Fusion Proteins**

E. coli ER2508 carrying plasmids encoding the MBP-peptide fusion proteins were grown in 2xYT-AG media for 8 h (250 rpm, 37°C). The cultures were subcultured in 2xYT-AG to achieve an OD₆₀₀ of 0.1 and grown
20 at 30°C overnight. This culture was used to inoculate a fermentor with medium of following composition (g/l): glucose (3.00); (NH₄)₂SO₄ 5.00; MgSO₄ • 7H₂O (0.25); KH₂PO₄ (3.00); citric acid (3.00); peptone (10.00); and yeast extract (5.00); pH 6.8.

 The culture was grown at 700 rpm, 37°C until the glucose from the
25 medium was consumed (OD₆₀₀ = ~6.0 – 7.0). Expression of the fusion protein was induced by the addition of 0.3 mM IPTG and the culture was grown for 2 h in fed-batch mode fermentation with feeding by 50% glucose at a constant rate of 2 g/l/h. The cells were removed from the medium by centrifugation. Samples of the cell pellet were analyzed by SDS-PAGE

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followed by the Western blot analysis using the mouse monoclonal antibody anti-E-Tag-HRP conjugate (Pharmacia) to visualize the expressed product.

D. Purification

5 The cell pellets were disrupted mechanically by sonication or chemically by treatment with the mild detergent Triton X-100. After removal of cell debris by centrifugation, the soluble proteins were prepared for chromatographic purification by dilution or dialysis into the appropriate starting buffer. The MBP fusions were initially purified either by amylose affinity chromatography or by anion exchange chromatography. Final
10 purification was performed using anti-E-Tag antibody affinity columns (Pharmacia). The affinity resin was equilibrated in TBS (0.025 M Tris-buffered saline, pH 7.4) and the bound protein was eluted with Elution buffer (100 mM glycine, pH 3.0). The purified proteins were analyzed for purity and integrity by SDS-PAGE and Western blot analysis according to standard
15 protocols.

For MBP fusions, IR agonist activity was observed for the Site 1-Site 1 dimer peptides shown in Table 10, below. Additional binding data for the MBP fusions are shown in Table 11, also below.

TABLE 10

Fus.	Monomer/ Linker	Sequence	SEQ ID NO.	Form.	Act.	Site IR	Fus. Conc.	MW (kDa)	K _d (HIR)
426	D8	MBP...NNNNGIEGRISIEFIEGR AQPAMA WLDQEWAWVQCEVYGRGCPAAAA (ETAG)AA	2132	6	N	2	0.76	52.2	1.4 x 10 ⁻⁶
429	D8-6aa-D8	MBP...NNNNGIEGRISIEFIEGRAQPAMAWLDQEWAWVQCEVYGRGCPGGSGGS KWLDQEWAWVQCEVYGRGCPAAAA(ETAG)AA	2133	6-6	N-N	2-2	3.2	55.3	1.3 x 10 ⁻⁶
430	H2C-4aa-RB6	MBP...NNNNGIEGRISIEFIEGRDYKDDDKFHENFYDWFVRQVSGGSLDALDRIM RYFEERPSLETAG	2134	1-6	A-	1-1	0.17	54.5	2.1 x 10 ⁻⁶
431	H2C-6aa-F8	MBP...NNNNGIEGRISIEFIEGRDYKDDDKFHENFYDWFVRQVSGGSHLCVLEE LFWGASLFGYCSGAAA(ETAG)AA	2135	1-4	A-N	1-2	3.3	54.8	4.7 x 10 ⁻⁸
432	H2C-12aa-F8	MBP...NNNNGIEGRISIEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2136	1-4	A-N	1-2	2.9	55.5	3.5 x 10 ⁻⁸
433	H2C-9aa-F8	MBP...NNNNGIEGRISIEFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGGSHLC VLEELFWGASLFGYCSGAAA(ETAG)AA	2137	1-4	A-N	1-2	2.8	55.2	2.1 x 10 ⁻⁸
434	G3-12aa-G3	MBP...NNNNGIEGRISIEFIEGRAQPAMARGGGTFYEFESALRKHGAGGGGGSG SGGSRGGGTFYEFESALRKHGAGAAA(ETAG)AA	2138	1-1	N-N	1-1	0.01	56	3.2 x 10 ⁻⁶
436	H2C-9aa-H2C	MBP...NNNNGIEGRISIEFIEGRAQPAMAFHENFYDWFVRQVSGGSGGSGFHEN FYDWFVRQVSA(ETAG)AA	2139	1-1	A	1-1	1.1	54.2	4.1 x 10 ⁻⁷
437	H2C	MBP...NNNNGIEGRISIEFIEGRAQPAMA FHENFYDWFVRQVSA(ETAG)AA	2140	1	N-N	1	0.3	51.5	8.3 x 10 ⁻⁶
427	G3-6aa-G3	MBP...NNNNGIEGRISIEFIEGRAQPAMARGGGTFYEFESTLRKHGAGGGGGSR GGGTFYEFESALRKHGAGAAA(ETAG)AA	2141	1-1	N-N	1-1	0.02	55.3	3.3 x 10 ⁻⁶
435	H2C-3aa-H2C- 3aa-H2C	MBP...NNNNGIEGRISIEFIEGRAQPAMAFHENFYDWFVRQVSGGSGFHENFYDWFV RQVSGGSGFHENFYDWFVRQVSA(ETAG)AA	2142	1-1-1	A-A-A	1-1-1	2.1	55.5	2.0 x 10 ⁻⁶
439	H2C-6aa-H2C	MBP...NNNNGIEGRISIEFIEGRAQPAMAFHENFYDWFVRQVSGGSGGSGFHEN FYDWFVRQVSA(ETAG)AA	2143	1-1	A-A	1-1	1.4	53.9	5.5 x 10 ⁻⁷
449	H2C-12aa-H2C	MBP...NNNNGIEGRISIEFIEGRAQPAMAFHENFYDWFVRQVSGGSGGSGGSA QPAMAFHENFYDWFVRQVSA(ETAG)AA	2144	1-1		1-1	1.5	51.8	6.2 x 10 ⁻⁷
452	G3	MBP...NNNNGIEGRISIEFIEGRAQPAMARGGGTFYEFESALRKHGAGAA A(ETAG)AA	2145	1		1	0.15	48.8	7.8 x 10 ⁻⁷
463	H2C-3aa-H2C	MBP...NNNNGIEGRISIEFIEGRAQPAMAFHENFYDWFVRQVSGGSGFHENFYDWFV	2146	1-1	A-A	1-1	1.8	50.1	9.6 x 10 ⁻⁷

		RQVSAAA(ETAG)AA						
464	LF-H2C	MBP...NNNNGIEGRISFIEGRDYKDDDK FHENFYDFWVRQVSAA(ETAG)AA	2147	1		0.045	48.4	3.9×10^{-8}
446	LF-F8	MBP...NNNNGIEGRISFIEGRDYKDDDKHLCVLEELFWGASLFGYCSGAAA(ETA GAA	2148	1		1.9	49.1	7.7×10^{-7}
459	SF-RB6	MBP...NNNNGIEGRISFIEGRDYKDDDKHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2149	3		0.069	48.1	7.7×10^{-8}
MB P*	lacZ	**		na		5.1	50	$> 1 \times 10^{-5}$

*MBP (negative control for the fusions) is fused to a small fragment of beta-galactosidase (lacZ); **MBP-lacZ fusion protein was derived from the plasmid pMal-c2 as purchased from NEB. Fus. = fusion; Act. = activity; Conc. = concentration; N = Antagonist; A = Agonist; LF = Long FLAG® epitope (DYKDDDDK; SEQ ID NO:177); SF = Short FLAG® epitope (DYKD; SEQ ID NO:1545); na = not applicable; Form. = formula; All dimers are linked C-N; Linker sequences are underlined.

TABLE 11

Fusion	Monomer/ Linker	Sequence	SEQ ID NO:	Form.	Site IR	High conc. tested (μM)	Kd (HIR) μM
431-	H2C-6aa-F8	MBP...NNNNGIEGRISFIEGRDYKDDDKFHENFYDFWVRQVSGGGSHLCVLEELFWGASLFGYCS GAAA(ETAG)AA	2150	1-6	1-2	0.2	0.033
431+	H2C-6aa-F8	DYKDDDKFHENFYDFWVRQVSGGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2151	1-6	1-2	0.2	0.0074
432-	H2C-12aa-F8	MBP...NNNNGIEGRISFIEGRDYKDDDKFHENFYDFWVRQVSGGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2152	1-6	1-2	0.2	0.02
432+	H2C-12aa-F8	DYKDDDKFHENFYDFWVRQVSGGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2153	1-6	1-2	0.2	0.0038
433-	H2C-9aa-F8	MBP...NNNNGIEGRISFIEGRDYKDDDKFHENFYDFWVRQVSGGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2154	1-6	1-2	0.2	0.03
433+	H2C-9aa-F8	DYKDDDKFHENFYDFWVRQVSGGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2155	1-6	1-2	0.2	0.004

The concentrations of these fusions vary depending on the expression quality. There are 2 sets of each fusion: uncleaved (-) and cleaved with factor Xa (+). The fusion proteins are in Tris buffer (20 mM Tris, 200 mM NaCl, 1 mM EDTA, 50 mM maltose, pH 7.5) and the cleaved fusions (+) are in the same Tris buffer (500 μl) + 12 μg Factor Xa. (Source of Factor Xa: New England Biolabs). Conc. = concentration; Form. = formula; All dimers are linked C-N; Linker sequences are underlined.

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E. BIAcore Analysis

For BIAcore analysis of fusion protein and synthetic peptide binding to insulin receptor, insulin (50 µg/ml in 10 mM sodium acetate buffer pH 5) was immobilized on the CM5 sensor chip (Flowcell-2) by amine coupling.

5 Flowcell-1 was used for background binding to correct for any non-specific binding. Insulin receptor (450 nM) was injected into the flow cell and the binding of IR to insulin was measured in resonance units (RUs). Receptor bound to insulin gave a reading of 220 RU. The surface was regenerated with 25 mM NaOH. Pre-incubation of receptor with insulin in a tube at RT

10 completely abrogated the response units to 16 RU. Thus, the system was validated for competition studies. Several maltose-binding fusion proteins, peptides, and rVabs were pre-incubated with insulin receptor before injecting over the insulin chip for competition studies. The decrease in binding/resonance units indicates that several MBP-fusion proteins can block

15 the insulin-binding site. The results are shown in Tables 12 and 13. The amino acid sequences referred to in the tables are identified in Figures 8 and 9A-9B, except the 447 and 2A9 sequences, which are shown below.

TABLE 12

BIAcore Results—Fusion Proteins Compete for Binding to IR

	Incubation Mixtures	Result (RUs)	Sequence Type
Controls	Insulin Receptor (IR) 450 nM	220	Positive Control
	Insulin (8.7 µM)	16	Negative Control
MBP Fus. Prots.	A7 (20A4)-MBP (4.1 µM) + IR	43	Formula 6 Motif
	D8-MBP (1.6 µM) + IR	56	Formula 6 Motif
	D10-MBP (3.4 µM) + IR	81	Formula 11 Motif
	447-MBP (11.5 µM) + IR	195	hGH Pept. Fus.
	MBP (13 µM) + IR	209	Negative Control

20

The A7 (20A4), D8, and D10 peptide sequence are shown in Figures 8 and 9A-9B. The 447 peptide sequence is: LCQRLGVGWPGWLSGWCA (SEQ ID NO:2156).

TABLE 13

25 BIAcore Results -- Synthetic peptides compete for binding to IR

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Incubation Mix	% Binding	Result (RUs)	Sequence Type
IR	100	128	Positive control
IR + 20D1	41	51.8	Formula 1 Motif
IR + D8	33	41.6	Formula 6 Motif
IR + 20C11	38	49	Formula 2 Motif (bkg high)
IR + H2	27	34.6	IGF (phosphorylated band)
IR + 2A9	100	128	IGF(bkg high)
IR + 20A4	33	41.8	Formula 6 Motif
IR + p53wt	97	124.5	P53 wild type

5 The concentration of each peptide was about 40 μ M and the concentration of IR was 450 nM. The 20D1, 20A4, and D8 peptide sequences are shown in Figures 8 and 9A-9B. The remaining peptide sequences are as follows: 447 = LCQRLGVGWPGWLSGWCA (SEQ ID NO:2156); 2A9 = LCQSWGVRIGWLTGLCP (SEQ ID NO:2157); 20C11 = DRAFYNGLRDLVGAVYGAWD (SEQ ID NO:1659); H2 = VTFTSAVFHENFYDWFVRQVS (SEQ ID NO:1784).

10 Regarding preparation of a Site 1 agonist comprising two D117 (H2C) peptides, a linker of only 3 amino acids (12 Å) provided a ligand of greater affinity for Site 1 of IR than a corresponding ligand prepared with a 9 amino acid (36 Å) linking region (Figure 29).

F. Stimulation of Autophosphorylation of IR by MBP-Fusion Peptides

15 MBP fusion peptides were prepared as described above, and then assayed for autophosphorylation of a insulin receptor construct transfected into 32D cells (Wang *et al.*, 1993; clone 969) (see Example, above). The results of these experiments shown in Figure 30 indicate that the H2C monomer and H2C-H2C homodimer peptides stimulate autophosphorylation of IR *in vivo*. H2C dimer peptides (Site 1-Site 1) with a 6 amino acid linker (H2C-6aa-H2C) were most active in the autophosphorylation assay. Other active dimer peptides are also shown in Figure 30, particularly H2C-9aa-H2C, H2C-12aa-H2C, H2C-3aa-H2C, and F8.

25 G. Insulin Receptor Binding Affinity and Fat Cell Potency of MBP-Fusion Peptides

Results of assays to determine binding affinity for insulin receptor and fat cell potency of the MBP-fusion peptides are shown in Table 14, below.

TABLE 14

Peptide	SEQ ID NO:	Formula	Site IR	Sequence	HIR Kd (mol/l)	FFC
RB426	2158	F6	2	MBP...NNNNLGIETRISFIEGR AQPAMA WLDEWAWVQCEVYGRGCPs AAA(ETAG)AA	1.4*10 ⁻⁶	
RB429	2159	F6-F6	2-2	MBP...NNNNLGIETRISFIEGRAQAPAMAWLDEWAWVQCEVYGRGCPsGGGSKWLDEWAWVQCEVYGRGCPsAAA(ETAG)AA	1.3*10 ⁻⁶	
RB505M	2160	F4	2	MBP...NNNNLGIETRISFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGAAA(ETAG)AA		
RB517M	2161	F4-F4	2-2	MBP...NNNNLGIETRISFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGSGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA		
RB515	2162	F4-F4	2-2	MBP...NNNNLGIETRISFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGSGSGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA		
RB510	2163	F4-F4-F4	2-2-2	MBP...NNNNLGIETRISFIEGRDYKDDDDK HLCVLEELFWGASLFGYCSGSGSGGS HLCVLEELFWGASLFGYCSGSGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA		
RB437	2164	F1	1	MBP...NNNNLGIETRISFIEGRAQAPAMA FHENFYDWFVRQVSAAA(ETAG)AA	8.3*10 ⁻⁶	
RB463	2165	F1-F1	1-1	MBP...NNNNLGIETRISFIEGRAQAPAMAFHENFYDWFVRQVSFGSFHENFYDWFVRQVSAAA(ETAG)AA	9.6*10 ⁻⁷	
RB439	2166	F1-F1	1-1	MBP...NNNNLGIETRISFIEGRAQAPAMA FHENFYDWFVRQVSFGSGSFHENFYDWFVRQVS-ETAG	5.5*10 ⁻⁷	
RB436	2167	F1-F1	1-1	MBP...NNNNLGIETRISFIEGRAQAPAMAFHENFYDWFVRQVSFGSGSGSFHENFYDWFVRQVSAAA(ETAG)AA	4.1*10 ⁻⁷	
RB449	2168	F1-F1	1-1	MBP...NNNNLGIETRISFIEGR AQPAMAFHENFYDWFVRQVSFGSGSGSGGS AQPAMAFHENFYDWFVRQVSAAA(ETAG)AA	6.2*10 ⁻⁷	
RB435	2169	F1-F1-F1	1-1-1	MBP...NNNNLGIETRISFIEGRAQAPAMAFHENFYDWFVRQVSFGSGSFHENFYDWFVRQVSAAA(ETAG)AA	2.0*10 ⁻⁶	
RB502	2170	F1	1	MBP...NNNNLGIETRISFIEGRDYKDDDDK VRVDWLQRNANFYDWFVAELVAAA(ETAG)AA		
RB508M	2171	F1-F1	1-1	MBP...NNNNLGIETRISFIEGRDYKDDDDKVRVDWLQRNANFYDWFVAELGGSGSGSGRVDWLQRNANFYDWFVAELGAAA(ETAG)AA		
RB509M	2172	F1-F1	1-1	MBP...NNNNLGIETRISFIEGRDYKDDDDKVRVDWLQRNANFYAWFVAELGGSGSGSGSGRVDWLQRNANFYDWFVAELGAA A(ETAG)AA		
RB452	2173	F1	1	MBP...NNNNLGIETRISFIEGRAQAPAMARGGGTFYEFWESALRKHGAGAAA(ETAG)AA	7.8*10 ⁻⁷	
RB427	2174	F1-F1	1-1	MBP...NNNNLGIETRISFIEGRAQAPAMARGGGTFYEFWESALRKHGAGGGSGSGGGTFYEFWESALRKHGAGAAA(ETAG)AA	3.3*10 ⁻⁶	
RB434	2175	F1-F1	1-1	MBP...NNNNLGIETRISFIEGRAQAPAMA RGGGTFYEFWESALRKHGAGGGSGSGSGSGGGTFYEFWESALRKHGAGAAA(ETAG)AA	3.2*10 ⁻⁶	

RB513	2176	F1	1	MBP...NNNNGIEGRISFIEGRDYKDDDDKGSLSDFYDWFERQLGKKAAA(ETAG)AA	
RB516	2177	F1-F1	1-1	MBP...NNNNGIEGRISFIEGRDYKDDDDKGSLSDFYDWFERQLGKKGGSGGSLDESFDWFERQLGKKAAA(ETAG)AA	
RB512	2178	F1-F1	1-1	MBP...NNNNGIEGRISFIEGRDYKDDDDKGSLSDFYDWFERQLGKKGGSGGSLDESFDWFERQLGKKAAA(ETAG)AA	
RB464	2179	F1	1	MBP...NNNNGIEGRISFIEGRDYKDDDDKGSLSDFYDWFERQLGKKGGSGGSLDESFDWFERQLGKKAAA(ETAG)AA	3.8*10 ⁻¹⁸
RB446	2180	F4	2	MBP...NNNNGIEGRISFIEGRDYKDDDDKHLVLEELFWGASLFGYCSGAAA(ETAG)AA	7.7*10 ⁻⁷
RB459	2181	F3	1	MBP...NNNNGIEGRISFIEGRDYKDDDDKHLVLEELFWGASLFGYCSGAAA(ETAG)AA	7.7*10 ⁻⁸
RB430	2182	F1-F3	1-1	MBP...NNNNGIEGRISFIEGRDYKDDDDKHLVLEELFWGASLFGYCSGAAA(ETAG)AA	2.1*10 ⁻⁶
RB430	2183	F1-F3	1-1	cleaved DYKDDDKFHENFYDWFVRQVSGGSLDALDRLMRYFEERPSLAA(ETAG)AA	-4*10 ⁻⁹
RB431	2184	F1-F4	1-2	MBP...NNNNGIEGRISFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGS HLCVLEELFWGASLFGYCSGAAA(ETAG)AA	4.7*10 ⁻⁸
RB431	2185	F1-F4	1-2	cleaved DYKDDDKFHENFYDWFVRQVSGGSGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	-8*10 ⁻⁹
RB432	2186	F1-F4	1-2	MBP...NNNNGIEGRISFIEGRDYKDDDKFHENFYDWFVRQVSGGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	3.5*10 ⁻⁸
RB432	2187	F1-F4	1-2	cleaved DYKDDDKFHENFYDWFVRQVSGGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	-6*10 ⁻⁹
RB433	2188	F1-F4	1-2	MBP...NNNNGIEGRISFIEGRDYKDDDK FHENFYDWFVRQVSGGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	2.1*10 ⁻⁸
RB508	2189	F1-F1	1-1	DYKDDDDKVRVDWLQRNANFYDWFVAELGGSGGSGRVDWLQRNANFYDWFVAELGAAAGAPVYPDPLEPSA	1.5*10 ⁻⁷
RB509	2190	F1-F1	1-1	DYKDDDDKVRVDWLQRNANFYDWFVAELGGSGGSGRVDWLQRNANFYDWFVAELGAAAGAPVYPDPLEPSA	5.5*10 ⁻⁸
RB505	2191	F4	2	DYKDDDDKHLVLEELFWGASLFGYCSGAAA(ETAG)AA	4.8*10 ⁻⁷
RB517	2192	F4-F4	2-2	DYKDDDDKHLVLEELFWGASLFGYCSGSGGSHLCVLEELFWGASLFGYCSGAAA(ETAG)AA	6.0*10 ⁻⁶
RB521	2193	F1-F1	1-1	MADYKDDDDKGSLSDFYDWFERQLGKKGGSGGSLDESFDWFERQLGKKAAA(ETAG)PG	4.4*10 ⁻⁸
RB535	2194	F1-F1	1-1	MADYKDDDDKGSLSDFYDWFERQLGKKGGSGGSGGSLDESFDWFERQLGKKAAA(ETAG)PG	~1.0*10 ⁻⁷
RB540	2195	F6	2	MADYKDDDDKMLDQEWAWVQCEVYGRGCPSSAAA(ETAG)PG	~1.0*10 ⁻⁷
RB539	2196	F6-F1	2-1	MADYKDDDDKMLDQEWAWVQCEVYGRGCPSSGSGGSLDESFDWFERQLGKKAAA(ETAG)PG	7*10 ⁻¹⁰
RB537	2197	F1-F6	1-2	MADYKDDDDKGSLSDFYDWFERQLGKKGGSGGSLDQEWAWVQCEVYGRGCPSSAAA(ETAG)PG	5.9*10 ⁻¹¹
RB538	2198	F1-F6	1-2	MADYKDDDDKGSLSDFYDWFERQLGKKGGSGGSGGSLDQEWAWVQCEVYGRGCPSSAAA(ETAG)PG	1.7*10 ⁻¹¹
RB626	2199	F6-F1	2-1	MADYKDEIAEWGRVRLVYGRVGGGSGGSGGSGGSLDESFDWFERQLGKKAAA(ETAG)PG	3.0*10 ⁻¹⁰

RB625	2200	F6-F1	2-1	MADYKDDDDKWLDQEWAWVQCEVYGRGCPSPPPDPDITTHRADPQGSLSDFYDWFERQLGKAAA(ETAG)PG	3.8×10^{-10}	++++
RB622	2201	F6-F1	2-1	MADYKDDDDKWLDQEWAWVQCEVYGRGCPSTPKPTPPPLSADGSLDSFYDWFERQLGKAAA(ETAG)PG	1.0×10^{-9}	+++
RB596	2202	F1	1	MQNDDGSLDSFYDWFERQLGHHHHHPG	9.4×10^{-8}	
RB569	2203	F1	1	MGSLDSFYDWFERQLGEEGGDHHHHHPG	2.1×10^{-7}	
RB570	2204	F1	1	MQNDDGSLDSFYDWFERQLGEEGGDHHHHHPG	2.5×10^{-8}	

ETAG = GAPVYPDPLEPR(SEQ ID NO:2205); MBP...NINNL = fusion junction to MBP at c-terminus of MBP; All dimers are linked C-N.

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Example 9: *In Vivo* Assays for Insulin Agonists

To test the *in vivo* activity of dimer peptide S519, an intravenous blood glucose test was carried out on Wistar rats. Male Mol:Wistar rats, weighing about 300 g, were divided into two groups. A 10 μ l sample of blood was taken from the tail vein for determination of blood glucose concentration. The rats were anaesthetized with Hypnorm/Dormicum at t = -30 min and blood glucose was measured again at t = -20 min and at t = 0 min. After the t = 0 sample was taken, the rats were injected into the tail vein with vehicle or test substance in an isotonic aqueous buffer at a concentration corresponding to a 1 ml/kg volume of injection. Blood glucose was measured at times 10, 20, 30, 40, 60, 80, 120, and 180 min. The Hypnorm/Dormicum administration was repeated at 20 min intervals. Results shown in Figure 33 demonstrate that the S519 (at 20 nmol/kg) peptide lowered blood glucose levels similar to levels observed for human insulin (at 2.5 nmol/kg) (n=8). The S519 peptide and human insulin showed comparable *in vivo* effects, both in magnitude and onset of response (Figure 33).

Example 10: IGF-1 Binding Peptides

Three major groups of peptide IGF-1-binding peptides were obtained from IGF-1R panning experiments: Site 1 A6 (FyxWF) (SEQ ID NO:1596); Site 1 B6 (FyxxLxxL) (SEQ ID NO:1732), and Site 2 (cysteine loops). See Beasley *et al.* International Application PCT/US00/08528, filed March 29, 2000, and Beasley *et al.*, U.S. Application Serial No. 09/538,038, filed March 29, 2000. Active peptides included 20E2 and RP6 (B6-like; Formula 2), S175 (A6-like; Formula 1), G33 (A6-like; Formula 1), RP9 (A6-like; Formula 1), D815 (Site 2), and D8B12 (Site 2) peptides. The IGF-1 binding peptides were analyzed by various assays, described as follows.

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A. Phage Competition

Phage competition studies were performed with Site 1 (RP9) and Site 2 (D815) monomer peptides. Plates were coated with IGF-1R (100 ng/well in carbonate buffer, pH 9.6) overnight at 4°C. Wells were blocked with 4% non-fat milk in PBS for 60 min at room temperature. One hundred microliters of rescued phage were added to each well. Peptides in varying concentrations were added and the mixtures were incubated for 2 h at room temperature. Plates were washed three times with PBS and 100 µl of anti-M13 antibody conjugated to horseradish peroxidase was added to each well. The labeled antibody was incubated at room temperature for 60 min. After washing, 100 µl of ABTS was added per well and the plates read in a microtiter reader at 450 nM.

Phage included RP9 (A6-like; Formula 1); RP6 (B6-like; Formula 2); D8B12 (Site 2); and D815 (Site 2). Peptides included RP9 and D815.

Peptide	Formula	Site IGF-1R	Sequence	SEQ ID NO:
D8B12	6	2	WLEQERAWIWCEIQGSGCRA	1884
D815	6	2	WLDQERAWLWCEISGRGCLS	2206
RP6	2	1	TFYSCLASLLTGTPQPNRGPOWERCR	1635
RP9	1	1	GSLDESFYDWFERQLG	1559

Results shown in Figures 34A-34E demonstrate that that RP9 and D815 peptides competed both Site 1 and Site 2 phage. These results illustrate the allosteric nature of the interaction with IGF-1R.

Phage competition studies were also performed with Site 2-Site 1 dimer peptides containing 6- or 12-amino acid linkers. Plates were coated with IGF-1R (100 ng/well in carbonate buffer, pH 9.6) overnight at 4°C. Wells were blocked with 4% non-fat milk in PBS for 60 min at room temperature. One hundred microliters of rescued phage were added to

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each well. Peptides in varying concentrations were added and the mixture incubated for 2 h at room temperature. Plates were washed three times with PBS and 100 μ l of anti-M13 antibody conjugated to horseradish peroxidase was added to each well. The labeled antibody was incubated for 60 min at room temperature. After washing, 100 μ l of ABTS was added per well and the plates read in a microtiter reader at 450 nM. Phage included RP9, RP6, D8B12, and D815. Peptides included D815-6L-RP9 and D815-12L-RP9. Linker sequences are underlined and shown below.

Peptide	Formula	Site IGF-1R	Sequence	SEQ ID NO:
D815-6L-RP9	6-1	2-1	LDQERAWLWCEISGRGCLSGGSGGSGSLDESFYDWFERQLGKK	2207
D815-12L-RP9	6-1	2-1	WLDQERAWLWCEISGRGCLSGGSGGSGGSGGSGSLDESFYDW FERQLGKK	2208

D8B12, D815, RP6, and RP9 amino acid sequences are shown in the previous section. Results shown in Figures 35A-35E demonstrate that dimers competed both Site 1 and Site 2 phage. This indicates that both dimer units were active at IGF-1R.

B. IGF-1 Proliferation Assays

FDC-P2 cells expressing the IL-3 and human IGF-1R receptors were grown in RPMI-1640 medium supplemented with 15% fetal bovine serum (FBS) and 5% WEHI conditioned medium (containing IL-3) in accordance with routine methods. Prior to an experiment, the cells were pelleted and washed two times in PBS. Following this, cells were resuspended in RPMI-1640 medium with 2% FBS and added to a 96-well plate at a concentration of 2×10^4 cells/well in 75 μ l. This was designated as the cell plate.

Peptides were suspended in PPMI-15% FBS (test medium). For the agonist assay, medium was added to rows 2-12 of a 96-well plate. The peptide was added to row 1 in 200 μ l of test medium at a final concentration of 60 μ M. The peptide was serially diluted (1:1) across rows 2-11. No

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peptide was added to row 12 (control; cells without IGF-1). For the antagonist assay, test medium containing 10 ng/ml IGF-1 (ED₅₀ test medium) was added to all wells of a 96-well plate. To row 1 was added 100 µl of peptide in ED₅₀ test medium at a concentration of 120 µM. The peptide was serially diluted (1:1) across rows 2-11. No peptide was added to row 12 (control; cells with IGF-1).

For both agonist and antagonist assays, 75 µl from the working plates was transferred to the appropriate rows in comparable cell plates. The starting peptide concentration for both agonist and antagonist assays was 30 µM. Each peptide was done in duplicate. Plates were incubated at 37°C for 45-48 h. Ten microliters of WST-1 (Cell Proliferation Reagent, Roche cat # 1 644 807) were added to each well and the plates were read in an ELISA reader (440/700 dual wavelength) each hour for 4 h. Graphs were prepared from the raw data using Sigma Plot. Peptides included:

Peptide	Formula	Site IGF-1R	Sequence	SEQ ID NO:
20E2	2	1	DYKDFYDAIDQLVRGSARAGGTRD	2209
D815	6	2	WLDQERAWLWCEISGRGCLS	2206
G33	1	1	GIISQSCPESFYDWFAGQVSDPWWCW	1600
RP6	2	1	TFYSCLASLLTGTPQPNRGPWERCRCR	1635
RP9	1	1	GSLDESFYDWFERQLG	1559
S175	1	1	GRVDWLQRNANFYDWFVAELG	1560

Results of the IGF-1 proliferation assays are shown in Figures 36-42. Figure 36 demonstrates that that peptides G33 (Site 1; ED₅₀ ~ 10 µM) and D815 (Site 2; ED₅₀ ~ 2 µM) showed agonist activity at IGF-1R, whereas peptides RP9 and RP6 showed no agonist activity. Figure 37 demonstrates that that peptides RP6 (Site 1; ED₅₀ ~ 1 µM) and RP9 (Site 1; ED₅₀ ~ 7 µM) showed antagonist activity at IGF-1R, whereas peptides G33 and D815 showed no antagonist activity. Figure 38 demonstrates that peptides S175

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and 20E2 exhibited weak agonist activity at IGF-1R ($ED_{50} > 10 \mu M$). Figure 39 shows that D815-RP9 dimers with 6- or 12-amino acid linkers acted as agonists at IGF-1R. Figure 40 shows that dimer peptide D815-6-G33 was inactive as an agonist at IGF-1R. Figure 41 shows that monomer peptide
5 RP6 acted as an antagonist at IGF-1R. The IGF-1 standard curve determined for FDC-P2 cells is shown in Figure 42.

The IGF-1R data for the Site 1 and Site 2 peptides is summarized in Table 15, below.

TABLE 15

Mon./Dimer	Form.	Site IGF-1R	Link.	Sequence	SEQ ID NO.	nM Ki app Kd	nM ED ₅₀ Growth	Max Action	nM IC ₅₀ Antag.	Ki/ ED ₅₀	Class
IGF-1			NA			0.69	0.30	100	2	2.3	A
rG33	1	1	NA	GIISQSCPESFYDWFAGQVSDPWWCW	1600	1450	500	>50	---	2.9	A
rD815	6	2	NA	WLDQERAWLWCEISGRGCLS	2206	4080	500	>50%	---	8.2	A
RP9	1	1	NA	GSLDESFYDWFERQLG	1559	417	---	<10%	900	0.5	N
D815-G33	6-1	2-1	6 aa	WLDQERAWLWCEISGRGCLSGGGSGGIISQSCPESF YDWFAGQVSDPWWCW	2210	624	---	<10%	nd		nd
D815-RP9	6-1	2-1	6 aa	WLDQERAWLWCEISGRGCLSGGGSGGSLDESFYDW FERQLGKK	2211	36	50	>50%	>500	0.8	A
D815-RP9	6-1	2-1	12 aa	WLDQERAWLWCEISGRGCLSGGGSGGGSGGSGSL DESFYDWFERQLGKK	2212	3	10,000	100	-----	0.0003	A

A = agonists; N = antagonist; nd = not determined; NA = not applicable; Form. = formula; Mon. = monomer; Antag. = antagonist; Link. = linker; Linker sequences are underlined.

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Example 11: Panning Peptide Libraries for IGF-1 binding proteins**A. Panning Secondary Libraries**

Soluble IGF-1R ("sIGF-1R") was obtained from R&D Systems. The soluble protein (> 95% pure) included the heterotetrameric (alpha 2-beta 2) extracellular domain of IGF-1R isolated from a mouse myeloma cell line. sIGF-1R (500 ng/well) was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, NUNC) and incubated overnight at 4°C. Wells were then blocked with MPBS (PBS buffer pH 7.5 containing 2% Carnation® non-fat dry milk) at room temperature (RT) for 1 h. Eight wells were used for each round of panning for the G33 and RP6 secondary libraries. The phage were incubated with MPBS for 30 min at RT, then 100 µl was added to each well.

For the first round, the input phage titer was 4×10^{13} cfu/ml. For rounds 2 and 3, the input phage titer was approximately 10^{11} cfu/ml. Phage were allowed to bind for 2 to 3 h at RT. The wells were then quickly washed 13 times with 200 µl/well of MPBS. Bound phage were eluted by incubation with 100 µl/well of 20 mM glycine-HCl, pH 2.2 for 30 s. The resulting solution was then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, then plated onto two 24 cm x 24 cm plates containing 2xYT-AG. The plates were incubated at 30° C overnight. The next morning, cells were removed by scraping and stored in 10% glycerol at -80° C. For subsequent rounds of affinity enrichment, cells from these frozen stocks were grown and phage were prepared as described above. A minimum of 72 clones was picked at random from the second, third, and fourth rounds of panning and screened for binding activity. DNA sequencing of the clones determined the amino acid sequences summarized in Figure 43A-43B.

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B. Panning Peptide Dimer Libraries

Microtiter plates were coated and blocked by standard methods, as follows. Plates were coated with sIGF-1R (see Example, above) or soluble IR (Bass construct; Bass *et al.*, 1996, *J. Biol. Chem.* **271**:19367-19375) in
5 0.2 M NaHCO₃, pH 9.4. One hundred microliters of solution containing either 50 ng IR or IGF-1R (rounds 1 and 2), 25 ng IR or IGF-1R (round 3), or 12.5 ng IR or IGF-1R (round 4) was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nalge NUNC) and incubated overnight at 4°C. Wells were then blocked with a solution of 2%
10 non-fat milk in PBS (MPBS) at RT for at least 1 h.

Eight wells coated with IR or IGF-1R were used for each round of panning. One hundred microliters of phage were added to each well. For the first round, the input phage titer was 3×10^{13} cfu/ml. For subsequent rounds, the input phage titer was approximately 10^{12} cfu/ml. Phage were
15 incubated for 2-3 h at RT. The wells were then quickly washed 13 times with 300 µl/well of PBS. Bound phage were eluted by incubation with 150 µl/well of 50 mM glycine-HCl, pH 2.0 for 15 min. The resulting solution was pooled and then neutralized with Tris-HCl, pH 8.0. Log phase TG1 cells were infected with the eluted phage, in 2xYT medium for 1 h at 37°C prior to
20 the addition of helper phage, ampicillin, and glucose (2% final concentration).

After incubation for 1 h at 37°C, the cells were spun down and resuspended in 2xYT-AK medium. The cells were then returned to the shaker and incubated overnight at 37°C. Phage amplified overnight were
25 then precipitated and subjected to the next round of panning. A total of 96 clones were picked at random from rounds 3 and 4 and screened for binding activity. Several clones from each pan were further tested for binding to IR or IGF-1R in phage ELISA by competition with soluble peptides as described in Beasley *et al.* International Application PCT/US00/08528, filed
30 March 29, 2000, and Beasley *et al.*, U.S. Application Serial No. 09/538,038,

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filed March 29, 2000. Competition was performed by addition of 5 μ l of RP9 peptide, recombinant D8 peptide, or both per well, followed by addition of 100 μ l of phage per well. Representative peptides are shown in Figures 44A-44B and in Table 16, below.

TABLE 16

Pep.	SEQ ID NO:	Form.	Site IR	Sequence	Description
RP27	2213	6-1	2-1	GLDQEAWECEVYGRGCPYGSLSDESFDWFERQLG	No linker
RP28	2214	6-1	2-1	RLEEWAWVQCEVYGRGCPSGGGSGSLDESFDWFERQLG	EEE Stretch in D8
RP29	2215	6-1	2-1	SLDREWACVKCEVYGRGCPGGGGSGSLDESFDWFERQLG	Repeat isolate
RP30	2216	6-1	2-1	SLEEEWAQVECEVYGRGCPSGGGSGSLDESFDWFERQLG	D8 by design
RP31	2217	6-1	2-1	SLEEEWAQVECEVYGRGCPSGGGSGSLDESFDWFERQLR	D8 & RP9 by design
RP32	2218	6-1	2-1	SIEEEWAQIKCDVWGRGCPPGGGSGSLDESFDWFERQLR	D8 & RP9 by design
RP33	2219	6-1	2-1	QLDLEWAWVQCEVYGRGCPGGGGSGSLDESFDWFERQLG	3 amino acid linker
RP34	2220	6-1	2-1	QLDEEWAGVQCEVYGRGCPSLDESFDWFERQLG	No linker
RP35	2221	6-1	2-1	RLEEEWRWVQCEVYGRGCAAGGGSGSLDESFDWFERQLG	EEE Stretch in D8
RP36	2222	6-10	2-1	SLDQEAWEVQCEVYGRGCPSGGGSGSDSDWAGYEWFEQLD	D8 (W1->S)- Group 6 by design

Pep. = peptide; Form. = formula; Linker sequences are shown in bold and underlined; All dimers are linked C-N

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C. Determination of Amino Acid Preferences

For both monomer and dimer peptides, amino acid preferences for each peptide were determined as follows. The expected frequency of each of the 20 amino acids at that position was calculated based on codon usage and % doping for that library. This was then compared to the actual frequency of occurrence of each amino acid at every position after four rounds of biopanning. Any amino acid that occurred at a frequency >2-fold was considered preferred. Most preferred amino acid(s) were those that have the greatest fold enrichment after panning. Preferred amino acid sequences for RP9, D8, and Formula 10 (Group 6) peptides are shown below.

TABLE 17

Peptide	Sequence	SEQ ID NO:
RP9	GSLDESFYDWFERQLG	1559
Regular	<u>GLA</u> DEDFYEWFERQLR L	2223
w/ Peptide	G <u>QL</u> DEDFYEWFDRLS A	2224
w/ Insulin	G <u>F</u> MDESFYEWFERQLR W A	2225

Table 17 shows preferred amino acid sequences for RP9 peptides. Residues in bold indicate strong preference; underlined residues indicate positions where more than one amino acid preference is seen. The first column indicates the conditions used for the panning procedure. "RP9" indicates sequence of the parent RP9; "Regular" indicates regular pan as described in methods for panning of random libraries; "w/ peptide" indicates panning in the presence of 2 nM RP9 peptide; "w/ insulin" indicates panning in the presence of 2 nM insulin.

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TABLE 18

Peptide	Sequence	SEQ ID NO:
D8 Parent:	WLDQEWAWVQCEVYGRGCPS	2129
Dimer Consensus	sLEEE EW aQIE CEVY /WGRGCps	2226
Monomer Consensus	sLEEE EW aQIq CEIY /WGRGCry W	1548

Table 18 shows preferred amino acid sequences for D8 peptides. Upper case residues in bold indicate strong preference (>90% frequency); upper case letters, non-bold, indicate some preference (5-15% higher frequency than expected); lower case letters indicate less preference (2-5% higher frequency than expected); similar preferences seen in D8 in both monomer and dimer libraries. The underlined Y/W indicates that both residues are equally preferred at that position. In the original D8 sequence that position is occupied by Y.

TABLE 19

Peptide	Sequence	Type	SEQ ID NO:
Group 6	W(A/E)GYEW(F/L)	preferred core	1549
Group 6	DSDWAGYEWFEELQD	preferred sequence	1595

Table 19 shows preferred amino acid sequences for Group 6 peptides. Underlined residues indicate preferred N-terminal and C-terminal extensions.

Example 12: Fluorescence-Based hIGF-1R Binding Assays

A. Heterogeneous Time-Resolved Fluorometric Assays

The effect of recombinant peptide G33 (rG33) on the binding of biotinylated-recombinant human IGF-1 (b-rhIGF-1) to recombinant human

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IGF-1R (rhIGF-1R) was determined using heterogeneous time-resolved fluorometric assays (TRF; DELFIA®, PE Wallac, Inc.). The rhIGF-1R protein included the extracellular domain of the receptor pre-propeptide, up to amino acid residue 932 (A. Ullrich *et al.*, 1986, *EMBO J.* 5:2503-2512).

- 5 Duplicate data points were collected at each concentration of competitor and the lines were designed to represent the best fit to a four-parameter non-linear regression analysis ($y = \min + (\max - \min) / (1 + 10^{((\log IC_{50} - x) * \text{Hillslope}))})$) of the data, which was used to determine IC_{50} values.

- The assay was performed using a 96-well clear microplate (NUNC MaxiSorp) with a final volume of 100 μ l. Microtiter plates were coated with 0.1 μ g rhIGF-1R in 100 μ l of $NaHCO_3$, pH 8.5 buffer, and incubated overnight at room temperature (RT). The plates were washed 3-times with 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl (TBS). This was followed by addition of 200 μ l blocking buffer (TBS containing 0.05% Bovine Serum Albumin (BSA, Cohn Fraction V)), and incubated for 1 h at RT. The plates were washed 6-times with a 1 X solution of Wallac's DELFIA® wash concentrate. Competitor was added in a volume of 50 μ l and serially diluted across the microtiter plate in TBS containing 0.05% BSA. Non-specific binding (background) was determined in the presence of 60 μ M hIGF-1.
- 10
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- Fifty microliters of b-rhIGF-1, 10 nM, diluted in TBS containing 0.05% BSA was added. The plates were incubated for 2 h at RT. After incubation, plates were washed 6-times with a 1X solution of Wallac's DELFIA® wash concentrate. Then the plates were treated with 100 μ L of Wallac's DELFIA® Assay Buffer containing a 1:1000 dilution of europium-labeled streptavidin and incubated for 2 h at RT. This was followed by washing 6-times with a 1 X solution of Wallac's DELFIA® wash concentrate. One hundred microliters of Wallac's DELFIA® enhancer was added, and the plates were shaken for 30 min at RT. After shaking, the fluorescence signal at 620 nm was read on a Victor² 1420 plate reader (PE Wallac, Inc.). Primary data were
- 25
30

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background corrected, normalized to buffer controls, and then expressed as % specific binding. The Z'-factor was greater than 0.5 ($Z' = 1 - (3\sigma_+ + 3\sigma_-) / |\mu_+ - \mu_-|$; Zhang *et al.*, 1999, *J. Biomol. Screen.* 4:67-73) and the signal-to-background (S/B) ratio was ~20. The results of these experiments are shown in Figure 45. The IC₅₀ value calculated for rG33 is shown in Table 20, below.

The effect of recombinant peptides D815 (rD815), RP9, D815-6aa-G33, D815-6aa-RP9, and D815-12aa-RP9 on the binding of b-rhIGF-1 to rhIGF-1R was determined using the fluorometric assay described above. IGF-1 was used as a control. Duplicate data points were collected at each concentration of competitor and the lines represent the best fit to a four-parameter non-linear regression analysis, which was used to determine IC₅₀ values. Results for rD815 are shown in Figure 46; results for RP9 are shown in Figure 47; results for D815-6-G33 are shown in Figure 48; results for D815-6-RP9 are shown in Figure 49; and results for D815-12-RP9 are shown in Figure 50; the results for IGF-1 are shown in Figure 51. The IC₅₀ values for the rD815, RP9, D815-6aa-G33, D815-6aa-RP9, and D815-12aa-RP9 peptides, and IGF-1 are shown in Table 20, below. Linker sequences are underlined.

20

TABLE 20

Competitor	Sequence	SEQ ID NO:	IC ₅₀ (M)
rG33	GIISQSCPESFYDWFAGQVSDPWWCW	1600	1.45×10^{-6} M
rD815	WLDQERAWLWCEISGRGCLS	2206	4.08×10^{-6} M
RP9	GSLDESFYDWFERQLG	1559	4.17×10^{-7} M
D815-6aa-G33	WLDQERAWLWCEISGRGCLSGGSGGSGIIS QSCPESFYDWFAGQVSDPWWCW	2210	6.24×10^{-7} M
D815-6aa-RP9	WLDQERAWLWCEISGRGCLSGGSGGSGSL DESFYDWFERQLGKK	2211	3.57×10^{-8} M
D815-12aa-RP9	WLDQERAWLWCEISGRGCLSGGSGGSGG SGGSGSLDESFYDWFERQLGKK	2212	3.22×10^{-9} M
IGF-1			6.85×10^{-10} M

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The order of potency of all peptides or dimers compared to IGF-1 was determined as: IGF-1 > D815-12aa-RP9 >> D815-6aa-RP9 > RP9 \cong D815-6aa-G33 > rG33 > rD815. These results suggest that the coupling of D815 with RP9 using an extended linker (12 versus 6 amino acids) produced a
5 potent competitor that approximates the affinity of IGF-1 for its own receptor.

B. Time-Resolved Fluorescence Resonance Energy Transfer Assays

The effect of Site 1 peptides, Site 2 peptides, and rhIGF-1 on the dissociation of biotinylated-20E2 (b-20E2, Site 1) from recombinant human
10 IGF-1R was determined using time-resolved fluorescence resonance energy transfer assays (TR-FRET). Best fit non-linear regression analysis of the data, was used to determine dissociation rate constants. Each data point represents a single observation.

The assay was performed using a 96-well white microplate (NUNC)
15 with a final volume of 100 μ l. Final incubation conditions were 16.5 nM b-20E2, 2.2 nM SA-APC (streptavidin-allophycocyanin), 2.2 nM Eu³⁺-rhIGF-1R (LANCETM labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA (Cohn Fraction V). Reactions were allowed to reach equilibrium for 6 h at RT. Following this, various peptides
20 or IGF-1 were added at a final concentration of 100 μ M or 30 μ M, respectively. The addition of peptides or IGF-1 initiated the measurement of dissociation (Time Zero, sec). The fluorescence signal at 665 nm was read on a Victor² 1420 plate reader (PE Wallac, Inc.) at 30 sec intervals.

Results of these experiments are shown in Figure 52. The buffer
25 controls did not vary over the time interval of study, which demonstrated that the equilibrium was not disturbed by the addition of diluent at Time zero. The addition of excess (> 1000-fold 20E2 K_d for IGF-1R) Site 1 peptides such as H2C, 20E2, or RP6 did not differ depending on specific the peptide used, and the dissociation rates of b-20E2 were similar for these peptides.
30 D8B12 (Site 2 peptide) and IGF-1 (binds both Site 1 and Site 2) did

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demonstrate significant differences in the rate of dissociation of b-20E2. This would suggest that these agents act as non-competitive or allosteric regulators of Site 1 binding.

The effect of various peptides or peptide dimers on the binding of biotinylated-20E2 (B-20E2) to recombinant human IGF-1R was determined using TR-FRET assays, described above. For these experiments, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis ($y = \min + (\max - \min) / (1 + 10^{((\log IC_{50} - x) * \text{Hillslope}))})$) of the data, which was used to determine IC_{50} values.

The assays were performed using a 384-well white microplate (NUNC) with a final volume of 30 μ l. Final incubation conditions were 15 nM b-20E2, 2 nM SA-APC, 2 nM Eu^{3+} -rhIGF-1R (LANCE™ labeled, PE Wallac, Inc.), 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, and 0.1% BSA (Cohn Fraction V). After 16-24 h of incubation at RT, the fluorescence signal at 665 nm and 620 nm was read on a Victor² 1420 plate reader (PE Wallac, Inc.). Primary data were background corrected, normalized to buffer controls, and then expressed as % specific binding. The Z'-factor was greater than 0.5 ($Z' = 1 - (3\sigma_+ + 3\sigma_-) / |\mu_+ - \mu_-|$; Zhang *et al.*, 1999, *J. Biomol. Screen.* 4:67-73) and the signal-to-background (S/B) ratio was ~ 4. Results of these experiments are shown in Figure 53. Table 21, below, shows the IC_{50} values calculated for these experiments. Notably, the C1 peptide showed IGF-1R affinities of ~1 nM (Figure 53) and ~10 nM (Table 21) in these assays.

TABLE 21

Competitor	Sequence	SEQ ID NO:	Formula	Site IGF-1R	IC_{50} (M)
C1	CWARPCGDAANFYDWFVQQAS	1550	1	1	8.80E-10
IGF-1					2.93E-09
RP9	GSLDESFYDWFERQLG	1559	1	1	3.93E-08

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20E2	DYKDFYDAIDQLVRGSARAGGTRD	2209	2	1	1.04E-07
E8	GGTVWPGYEWLRNA	2118	10	2	2.53E-07
H2C	FHENFYDWFVQRVSKK	2117	1	1	4.60E-07
S173	LDALDRLMRYFEERPSL	1830	3	1	6.29E-06
D8B12	WLEQERAWIWCEIQGSGCRA	1884	6	2	1.13E-05
A6	SAKNFYDWFVKK	1551	1	1	3.10E-05

C. Fluorescence Polarization Assays

The effect of various peptide monomers and dimers on the binding of fluorescein-RP9 (FITC-RP9) to soluble human insulin receptor-immunoglobulin heavy chain chimera (sIR-Fc; Bass *et al.*, 1996, *J. Biol. Chem.* **271**:19367-19375) was determined using fluorescence polarization assays (FP). For these experiments, each data point represents the average of two replicate wells. The lines represent the best fit to a four-parameter non-linear regression analysis of the data, which was used to determine IC₅₀ values.

The assays were performed in a 384-well black microplate (NUNC) with a final volume of 30 μ l. Final incubation conditions were 1 nM FITC-RP9, 10 nM sIR, 0.05 M Tris-HCl (pH 8 at 25°C), 0.138 M NaCl, 0.0027 M KCl, 0.05% BGG (bovine gamma globulin), 0.005% Tween-20[®]. After 16-24 h of incubation at RT, the fluorescence signal at 520 nm was read on an Analyst[™] AD plate reader (LJL BioSystems, Inc.). Primary data were background corrected using 10 nM sIR without FITC-RP9 addition, normalized to buffer controls and then expressed as % specific binding. The Z'-factor was greater than 0.5 ($Z' = 1 - (3\sigma_+ + 3\sigma_-) / |\mu_+ - \mu_-|$; Zhang *et al.*, 1999, *J. Biomol. Screen.* **4**:67-73) and the assay dynamic range was ~125 mP. In parallel with these experiments, TR-FRET assays were performed using rhIGF-1R and b-20E2, as described above. Results of the FP and TR-FRET experiments are shown in Table 22, below.

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TABLE 22

Peptide	FP sIR-Fc	TR-FRET rhIGF-1R	Bndg Ratio IGF-1R / IR	Form.	Site IGF-1R	SEQ ID NO:	Sequence
RP4	17	8100	476	2	1	1552	PPWGARFYDAIEQLVFDNL
S175	10	1650	165	1	1	1560	GRVDWLQRNANFYDWFVA ELG
RP15	28	706	25	1	1	2130	SQAGSAFYAWFDQVLRTV
H2C (D117)	66	600	9	1	1	2117	FHENFYDWFVQRVSKK
20E2 (D118)	51	100	1.9	2	1	2209	DYKDFYDAIDQLVRGSARA GGTRD
RP9	24	33	1.4	1	1	1559	GSLDESFYDWFERQLG
G33	139	178	1.3	1	1	1600	GIISQSCPESFYDWFAGQV SDPWWCW
E8 (D120)	206	175	0.85	10	2	2118	GGTVWPGYEWLRNA
C1 (D112)	52	10	0.19	1	1	1550	CWARPCGDAANFYDWFV QQAS
RP16	6400	961	0.15			1553	VMDARDDPFYHKLSELVT

5 FP sIR-Fc column shows IC₅₀ (nM) values obtained (vs. FITC-RP9); TR-FRET rhIGF-1R column shows IC₅₀ (nM) values obtained (vs. b-20E2); for binding ratio: higher values indicated higher affinity for IR than IGF-1R. Form. = formula; Bndg. = binding.

10 These results demonstrated that S175, RP4, and RP15 showed high affinities for IR and showed high binding ratios for IGF-1R over IR. H2C, 20E2, RP9, and C1 were slightly less potent than S175, RP4, and RP15 at IR, and these peptides had lower binding ratios for IGF-1R over IR. G33 and E8 were less potent than S175, RP4, and RP15 at IR, and showed comparable binding to IGF-1R and IR. RP16 had poor potency at IR and IGF-1R, but had higher affinity for IGF-1R than IR.

Example 13: IGF-1R Binding Peptides – Additional Isolates

15 The isolation and characterization of peptides which bind to and subdivide the insulin receptor binding site into multiple, non-overlapping regions designated Site 1 and Site 2 has been previously described (Beasley *et al.*, U.S. Application Serial No. 09/538,038, filed March 29, 2000, published as WO 01/72771; Pillutla *et al.*, U.S. Patent Application Serial No.

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09/962,756, filed September 24, 2001; Pillutla *et al.*, 2002, *J. Biol. Chem.* 277:22590-22594). To identify IGF-1R antagonists, a multi-tiered approach was used. First, Site 1 peptides with greater selectivity for IGF-1R as compared to IR were identified. Second, secondary libraries were generated using information from the primary library pannings. These secondary libraries were designed to define the amino acid requirements for binding, specificity, and affinity.

To determine optimal sequence requirements within the motif, a secondary library based on a clone identified from the random library was made where the flanking regions were held constant, while the core was allowed to change. The library was prepared from doped oligonucleotides so that half of the amino acid residues (on average) in the core sequence were altered per peptide. Panning of these libraries identified substitutions within the core that did or did not affect binding. In an alternative approach, amino acids in the flanking regions conferring binding affinity and/or specificity were defined by designing secondary libraries wherein the core was held constant and the flanking sequences were either doped or randomized. For both types of libraries, amino acids optimal for binding were selected by panning against IGF-1R. Once secondary peptides with the appropriate binding characteristics were identified, a preferred peptide was defined. To do this, the amino acids at each position were optimized based on a comparison of the expected results from the doping strategy and the actual results observed in the binding population.

A. Primary Peptide Libraries

The *E. coli*, strain TG1 (genotype = K12Δ(*lac-pro*), *supE*, *thi*, *hsdΔ5/F'*[*traD36*, *proAB*, *lacI^f*, *lacZΔM15*]) was obtained from Pharmacia (Piscataway NJ). DNA fragments coding for peptides containing 40 random amino acids were generated by a PCR-technique using synthetic oligonucleotides. A 145-base oligonucleotide was synthesized to include the sequence (NNK)₄₀ where N = A, C, T, or G and K = G or T. This

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oligonucleotide was used as the template in PCR reactions along with two shorter oligonucleotide primers, both of which were biotinylated at their 5' ends. The resulting product was purified, concentrated, and ligated to the phagemid pCANTAB5E (Pharmacia). The ligation product was purified and
5 electroporated into competent bacterial cells. The transformants were grown at 37°C for 1 h, pooled and plated onto selection medium. Depending upon the amount of DNA electroporated, the diversity of the random 40mer peptide cell library was found to be between 1.6×10^{10} and 1×10^{11} independent clones. The phage library was produced by rescue of the cell
10 library according to standard phage preparation protocols (G.P. Smith and J.K. Scott, 1993, *Methods Enzymol.* **217**:228-257). Phage titers were usually at 4×10^{13} cfu/ml. In previous experiments, sequencing of randomly selected clones from the cell library indicated that about 54% of all clones were in-frame. The short FLAG sequence (DYKD; SEQ ID NO:1545), was
15 included at the N-terminus as an immunoaffinity tag. In addition, the E-tag epitope (GAPVPYPDPLEPR; SEQ ID NO:XX) was engineered into the carboxy terminus of the peptide. Additional random phage libraries of 20mer peptides were constructed using a similar approach. The diversity of these cell libraries was estimated to be $> 1.1 \times 10^{11}$ clones.

20 **B. Secondary and Tertiary Libraries**

The desired number of amino acid mutations were introduced in the parental peptide at the codon level when the synthetic DNA template was produced. For example, where a change in 45% of the amino acids was desired (i.e., 9 changes/20 amino acids), then a 60% change at the codon
25 level was needed due to the redundancy of the genetic code (efficiency factor of 0.75). Per position, this translated to 20% doping at the level of DNA synthesis. At the DNA synthesis level, a 20% doping included the following ratio of nucleotides in the synthetic template:

A 80% A, 6.6% C, 6.6% G, 6.6% T

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<u>C</u>	6.6% A	80% C,	6.6% G,	6.6% T
<u>G</u>	6.6% A	6.6% C,	80% G,	6.6% T
<u>T</u>	6.6% A	6.6% C,	6.6% G,	80% T

In this chart, the **A**, **C**, **T**, **G** (underlined and in bold) bases represent the original bases in the parental sequence. When the clones from cell libraries were sequenced and the number of amino acid mutations was examined per peptide, the average number of changes was found to correlate to the desired value. After the synthetic template was obtained, the DNA was ligated to the pCANTBA5E phagemid vector to produce the cell library in the TGI strain as previously described. Phage rescue was carried out to produce the phage library used in the panning experiments.

C. Panning of peptide libraries

A standard method was used to coat and block all microtiter plates. Plates were coated with IGF-1R in 0.2 M NaHCO₃, pH 9.4. One hundred microliters of solution containing 100 ng of IGF-1R was added to an appropriate number of wells in a 96-well microtiter plate (MaxiSorp plates, Nunc) and incubated overnight at 4°C. Wells were then blocked with a solution of 2% non-fat milk in PBS (MPBS) at room temperature (RT) for at least 1 h.

Four to eight wells coated with IGF-1R were used for each round of panning. One hundred microliters of phage were added to each well. For the first round, the input phage titer was $\sim 10^{13}$ cfu/ml. For subsequent rounds, the input phage titer was approximately 10^{12} cfu/ml. Phage were allowed to bind for 2-3 h at RT. The wells were then quickly washed 13 times with 300 μ l/well of PBS. Bound phage were eluted by incubation with 150 μ l/well of 50 mM glycine-HCl, pH 2.0 for 5 min. The resulting solution was pooled and then neutralized with Tris-HCl, pH 8.0.

Log phase TG1 cells were infected with the eluted phage, in 2xYT medium for 1 h at 37°C prior to the addition of helper phage, ampicillin and glucose (2% final concentration). After incubation for another hour at 37°C,

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the cells were spun down and resuspended in 2xYT-AK medium. The cells were then returned to the shaker and incubated overnight at 37°C. Phage amplified overnight was then precipitated and subjected to the next round of panning. A total of 96 clones were picked at random from rounds 3 and 4 and screened for binding activity.

D. ELISA Analyses of Phage

For phage pools, cells from frozen stocks were grown and phage were prepared as described above. For analysis of individual clones, colonies were picked and phage prepared as described above. Subsequent steps were the same for pooled and clonal phage. Microtiter wells were coated and blocked as described above. Wells were coated with either IGF-1R or IR. Phage resuspended in MPBS (PBS containing 2% non-fat milk) were added to wells (100 µl/well) and incubated at room temperature for 1 h. The phage solution was then removed, and the wells were washed three times with PBS at room temperature.

Anti-M13 antibody conjugated to horseradish peroxidase (Pharmacia Biotech) was diluted 1:3000 in MPBS and added to each well (100 µl/well). Incubation was for another hour at room temperature, followed by PBS washes as described. Color was developed by addition of ABTS solution (100 µl/well; Boehringer). Color development was stopped by adjusting each well to 0.5% SDS. Plates were analyzed at 405 nm using a SpectraMax 340 plate reader (Molecular Devices) and SoftMax Pro software. Data points were averaged after subtraction of appropriate blanks. A clone was considered "positive" if the A_{405} of the well was ≥ 2 -fold over background.

E. Determination of Amino Acid Preferences

Amino acid preferences for each peptide were determined as follows. The expected frequency of each of the 20 amino acids at that position was

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calculated based on codon usage and % doping for that library. This was then compared to the actual frequency of occurrence of each amino acid at every position after four rounds of biopanning. Any amino acid that occurred at a frequency ≥ 2 -fold was considered preferred. The most preferred amino acid(s) were defined as those with the greatest enrichment after panning. Using the amino acid preferences determined for each position, peptides with the most preferred sequences were designed.

Representative monomer and dimer peptides identified by panning secondary libraries for binding to IGF-1R are shown in Figures 54A-54B, 55A-55B, 56A-56B, 57A-57B, 58A-58B, 59A-59B, 60A-60C, 61A-61B, 62A-62B, 63A-63B, and 64A-64B. Primary library pannings produced several peptides, including RP6, RP48, RP52, RP54, RP56, and RP60, described above. Peptides designed according to amino acid preferences (i.e., peptides by design) included RP30-IGF, RP31-IGF, and RP33-IGF.

Example 14: IGF-1 Antagonist Peptides

A. Cells and Reagents

MCF-7 and MiaPaCa cell lines were obtained from the American Type Culture Collection (Manassas, VA). Cells were routinely grown in RPMI1640 medium supplemented with 10% fetal bovine serum and 1% glutamax. The extra-cellular domain of IGF-1R was obtained as a recombinant protein from R&D Systems (Minneapolis, MN).

B. Whole-cell lysates

For qualitative IRS-1 phosphorylation analysis, MCF-7 cells in monolayer cultures (about 80% of confluency) were used. After about 20 h of starvation in serum-free RPMI medium (GibcoBRL), cells were stimulated for 10 min in the same medium containing IGF-1 (Peprotech), or IGF-1 plus peptides (synthetic peptides produced by Research Genetics), or no addition as a negative control. After treatment, cells were rinsed twice with ice-cold PBS containing 0.2 mM PMSF and 1 mM Na_3VO_4 (all from SIGMA).

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Cells were scraped into the same buffer and pelleted by centrifugation at 200 x g for 3 min. Lysis was done in RIPA buffer (0.8766% NaCl, 0.11% SDS, 0.5% deoxycholic acid (all from SIGMA), 1% Triton X-100, (Boehringer Mannheim)) containing phosphatase inhibitor cocktails 1 and 2 (SIGMA) and
5 protease cocktail inhibitor tablet (Boehringer Mannheim) for 5 min on ice. Cell lysates were cleared by centrifugation for 5 min at 14 000 x g and the resulting supernatant was snap-frozen in ethanol-dry ice and stored at – 80° C. The protein concentration was determined using the D_C Protein Assay Kit (Bio-Rad Laboratories).

10 **C. Immunoprecipitation and Western Blot Analysis**

Immunoprecipitations were performed with pre-cleared lysates for 4 h at 40° C using 0.3-0.5 mg total protein with 1 µg polyclonal anti-IRS-1 antibody (Upstate Biotechnology), and 25 µl protein A/agarose slurry (SIGMA). Agarose beads with immobilized proteins were washed 3 times
15 with IP wash buffer (50 mM Tris pH 7.5 (GibcoBRL), 150 mM NaCl, 1 mM Na₃VO₄, 0.2 mM PMSF). Protein elutions and denaturation were done for 3 min at 95° C in 30 µl of Laemmle sample buffer (Bio-Rad Laboratories) with 0.5 M β-mercaptoethanol (SIGMA).

Immunoprecipitates were subjected to SDS-PAGE on 4-15% Tris-HCl
20 Ready Gels and transferred to Trans-Blot Transfer Medium nitrocellulose membranes (both from Bio-Rad Laboratories). Membranes were blocked with PBS-Tween 20 (SIGMA) containing 2% non-fat milk. For detection of IRS-1 protein, blots were incubated with anti-IRS-1 antibody, followed by secondary antibody goat anti-rabbit IgG, HRP-conjugate. For detection of
25 phosphorylated IRS-1, blots were incubated with monoclonal anti-phosphotyrosine (4G10) HRP-conjugated antibody. All antibodies were obtained from Upstate Biotechnology. Blots were exposed to an enhanced chemifluorescence substrate (ECL Western Blotting Analysis System, Amersham Pharmacia Biotech). Films were developed and fluorescent
30 signal was visualized for qualitative analysis.

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D. MCF-7 and MiaPaCa Cell Assays

Peptides produced synthetically were maintained as 30 mM stock in 100% DMSO, while recombinant dimers were diluted in water. All synthetic and recombinant peptides were stored at -80°C. The final concentration of DMSO was < 0.1%. MCF-7 and MiaPaCa (ATCC, Rockville, MD) cells were maintained in RPMI containing 10% FBS. All cells were starved overnight by growing them in RPMI media, which was serum free. Cells were trypsinized, washed twice with PBS before being seeded at $1-3 \times 10^3$ cells per well in a 96-well plate with a volume of 150 μ l/well. All points were done in duplicate in 96-well plates. For antagonist activity assays, immediately before the addition of peptides, all media was gently removed from the wells. Peptides were serially diluted 1:2 in a final volume of 150 μ l in a separate plate using RPMI containing 0.1% FBS plus 50 nM IGF-1. This mixture was transferred onto the cells, and the plates were incubated for 72 h at 37°C in a CO₂ incubator. To quantitate cell number, 10 μ l of WST-1 reagent (Roche Molecular Biochemicals, Indianapolis, IN) was added to each well and the plates were returned to the 37°C/CO₂ incubator for approximately 2 h. Measurements were then read at 440 nm, with 700 nm used as a reference.

E. Binding (ALPHAScreen) Assays

To assay binding, the relative potencies of peptides as compared to IGF-1 were analyzed in a competition system utilizing biotinylated-human IGF-1 (b-hIGF-1) and His-tagged soluble recombinant human IGF-1R (srhIGF-1R-his; R&D systems, Inc., Minneapolis, MN). Detection of the receptor ligand interaction was measured in an amplified luminescent proximity homogeneous assay (ALPHAScreen; BioSignal-Packard, Montreal). The assay was performed in 384-well Nunc™white polystyrene microplates (Nalge Nunc International, Naperville, IL) with a final volume of 40 μ l. Final incubation conditions were 1 nM b-hIGF-1, 10 nM srhIGF-1R-

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his, 0.025 M HEPES (pH 7.4 at 25°C), 0.100 M NaCl, 0.1% BSA (Cohn Fraction V; Sigma Chemical Co., St. Louis, MO), 10 µg/ml nickel conjugated acceptor beads, and 10 µg/ml streptavidin conjugated donor beads.

For the first step of the assay, hIGF-1 (PeproTech, Inc., Rocky Hill, NJ), b-hIGF-1 (see below), and peptides were incubated for 2 h at room temperature. Each concentration of competitor was assayed in duplicate. Non-specific binding was determined in the presence of 3×10^{-5} M hIGF-1. In the second step of the assay, acceptor beads were added and the incubation was continued for 0.5 h. In the final step, donor beads were added and the incubation was continued for an additional 1 h. At the end of the incubation period, the fluorescence signal at 520 nm was read on a Fusion-α HT plate reader (Packard BioScience Company, Meriden, CT). Primary data were background corrected, normalized to buffer controls, and then expressed as % specific binding. The data were fit to a four-parameter non-linear regression analysis ($y = \min + (\max - \min) / (1 + 10^{((\log IC_{50} - x) * \text{Hillslope}))}$), which was used to determine IC_{50} values. The Z'-factor for this assay was greater than 0.7 ($Z' = 1 - (3\sigma^{++} + 3\sigma^{-}) / |\mu^{+} - \mu^{-}|$) and the signal-to-background (S/B) ratio was between 40 and 70.

Human IGF-1 was biotinylated on free amino groups using Pierce EZ-Link™ Sulfo-NHS-LC-Biotinylation Kit (PN #21430, Pierce, Rockford, IL). Human IGF-1, at 2 mg/ml in PBS, pH 7.2, was incubated at room temperature for 30 min with a 20-fold excess of sulfo-NHS-LC-biotin over theoretical total free amino groups. Unreacted biotins were removed by extensive dialysis (Pierce Slide-A-Lyzer® Dialysis Cassettes) against PBS, and degree of conjugation was determined by HABA (2-(4'-hydroxyazobenzene) benzoic acid) assay (Pierce product literature #21430). Number of biotins per hIGF-1 varied between 3 and 5.

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F. FDC-P2 Cell Assays

Peptides produced synthetically were maintained as 30 mM stock in 100% DMSO, while recombinant dimers were diluted in water. All synthetic and recombinant peptides were stored at -80°C. The final concentration of DMSO was < 0.1%. FDC-P2 (obtained from Dr. J. Pierce, National Institutes of Health, Bethesda, MD) cells were maintained in RPMI containing 15% FBS and 5% WEHI (Genoquest, Germantown, MD) at 37°C in a CO₂ incubator. To initiate experiments, all cells were starved for 5 h in RPMI containing 1% FBS. Cells were seeded at 1×10^4 cells per well into 96-well plates at a volume of 75 µl/well. Peptides were added at 2X final concentrations and all points were done in duplicate. For antagonist assays, peptides at 2X concentration were serially diluted 1:2 in a final volume of 75 µl in a separate plate using RPMI containing 0.1% FBS and 1 nM IGF-1. This mixture was transferred onto the cells to yield a final volume of 150 µl. The plates were incubated for 48 h at 37°C in a CO₂ incubator. To quantitate cell number, 10 µl of WST-1 reagent (Roche Molecular Biochemicals, Indianapolis, IN) was added to each well and the plates were returned to the 37°C/CO₂ incubator for approximately 2 h. Measurements were then taken at 440 nm, with 700 nm used as a reference.

G. Results

Peptide RP33-IGF exhibited an affinity for IGF-1R close to that of IGF-1 (9 nM; Table 23). Other peptides, such as RP54 showed affinity in the micromolar range (Table 23). In contrast to the observations made for IR, competition experiments indicated that IGF-1R Site 1 and 2 peptides were able to compete with each other. This suggested that the functional interactions between Site 1 and Site 2 in IGF-1R differed from those found in IR (unpublished data).

To determine if any Site 1 peptides could act as antagonists, proliferation assays were established utilizing IGF-1 and IGF-2 responsive

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human tumor cell lines. Sixteen human tumor cell lines were screened for their ability to proliferate in the presence IGF-1 and IGF-2 under serum-free conditions. Two cell lines, MCF-7 (breast carcinoma) and MiaPaCa (pancreatic carcinoma), showed the best dose response curves for IGF-1
5 (ED₅₀ = 5 nM; Figures 65A-65F), and were used for subsequent experiments.

Peptides were synthesized and screened in the proliferation assay at an IGF-1 dose ten times the ED₅₀ (50 nM). Several antagonist peptides were identified, including RP33-IGF, which consistently blocked IGF-1 and
10 IGF-2 proliferation of both MCF-7 and MiaPaCa (Figures 66B-66C). In addition, peptides RP52 and RP54 were found to act as antagonists in at least one cell line (Table 26; Figures 70A-70B). Peptides RP52 and RP54 are classified as miscellaneous peptides, which were not categorized into any of the formulae (e.g., Formula 1, Formula 2, etc.) disclosed herein.

15 Experiments were then performed to determine whether antagonist peptides could block receptor activation at the level of key signaling intermediate, IRS-1. First, the optimal time and concentration of IGF-1 needed for maximal activation of IRS-1 was established (Figures 67A-67B and Figures 68A-68B). Maximum phosphorylation of IRS-1 was observed
20 after 10 min of treatment and was followed by a drop-off of the signal (Figures 67A-67B). This pattern was presumably due to degradation of the IRS-1 protein by a mechanism involving proteasomes (Lee *et al.*, 2000, *Mol. Cell. Biol.*, 2000, 20:1489-1496). Second, RP33-IGF was compared to two unrelated peptides. The RP33-IGF peptide inhibited IRS-1 phosphorylation,
25 whereas the unrelated peptides had no effect in the proliferation assay (Figures 69A-69B).

The RP6KK peptide was also tested for activity, since the RP33-IGF peptide was originally derived from the RP6KK sequence. Both RP6KK and RP33-IGF were found to effectively block activation of IRS-1 by IGF-1
30 (Figures 69A-69B). At the concentration used, greater than 90% of the protein was unphosphorylated, indicating that both peptides efficiently

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blocked IGF-1R activation. However, RP33-IGF differed from RP6KK by 11 amino acids, and RP33-IGF was a superior IGF-1R antagonist in the cell proliferation assays (Tables 24-25). The difference in biological activity did not appear to be related to stability of the peptides since both were found to remain intact during the course of the assays (unpublished data).

5

TABLE 23

Pep. (Clone)	Sequence	Site*	Formula	Affin. (μM)	Activ. [§]	IC ₅₀ (μM)
RP33-IGF	SFYS[C]LESLVNGPAEKSRGQWDG[C]RKK SEQ ID NO:2232	1	2	0.009	Antag.	0.1 - MCF-7 0.7 - MiaPaCa
RP6KK	TFYS[C]LASLLTGTTPQPNRGPWERC[R]KK SEQ ID NO:2233	1	2	0.19	Antag.	
RP52 (20C-3-A3-IGFR)	EISFR[C]QLFVLAGMHP[C]PVDVGGEGFE SEQ ID NO:2245		Misc.	ND	Antag.	0.5 - MCF-7
RP54 (20C-4-A7-IGFR)	EGSSI[C]NLLARAQIVELAL[C]EMGVQEE SEQ ID NO:2246		Misc.	1.6	Antag.	2.5 - MCF-7 4.3 - MiaPaCa

Peptide antagonists of IGF-1R identified from primary and secondary library panning. *site to which a peptide binds was assigned based on competition assays using both IR and IGF-1R as target; [§]affinity was determined using the AlphaScreen assay versus IGF-1 as described herein; antagonistism was determined from the proliferation assays in the presence of IGF-1 using MCF-7 and MiaPaCa cells as described herein; ND = not done; Pep. = peptide; Affin. = affinity; Antag. = antagonist; Misc. = miscellaneous peptide; Cysteine residues are boxed.

TABLE 24

IGF-1R Antagonists in MCF-7 Cells

Peptide	Form.	Site IGF-1R	Cellular IC ₅₀ (M)	Binding IC ₅₀ (M)	Sequence
H2C-A-H6	1	1	4.0E-07	3.2E-05	VGRASGFPE FD WF GRQLSLQSGEQ SEQ ID NO:2228
C1KK	1	1	2.8E-06	4.2E-08	DYKDCWARPCGDAAN FD WF VQQASKK SEQ ID NO:2266
RP33K-IGF	2	1	2.1E-08	1.8E-09	SD WF SC ES VNGPAEKSRGQWDGCRK SEQ ID NO:2266
RP6KK	2	1	9.0E-06	1.8E-07	TD WF SC AS LTGTPQPNRGPWERCKK SEQ ID NO:2233
RP54	Misc.		4.3E-06	4.9E-07	EGSSICNLLARAQIVELALQEMGVQEE SEQ ID NO:2246
RP52	Misc.		4.5E-07	3.0E-05	EISFRQQLFVLAGMHPCVPDVGGEGFE SEQ ID NO:2245
RP30-IGF-12- RP30-IGF	2-2	1-1	3.4E-06	2.4E-07	ADYKDS SD WF SC ES VNGGAERSDQWEGCRGGSGGGSS SD ES VNGGAERSDQWEGCRAAAG APVPYPDPLEPRPG; SEQ ID NO:2241
D8B12-12-RP9	6-1	2-1	6.9E-06	N/A	ADYKDWLEQERAWIWCEKSGCRAGSGGGSGGGSDDDKGSIDES SD WF ERQLGKAAAGAPVPYPDPLE PRG; SEQ ID NO:2244

Monomer and dimer peptides which block IGF-1 activity in cell proliferation assays in MCF-7 cells. Form. = formula; Misc. = miscellaneous sequence; Cysteine pairs are shaded and underlined; FY, WF, and L residues from Formula 1 and Formula 2 motifs are shaded and shown in bold.

TABLE 25

IGF-1R Antagonists in MiaPaCa Cells

Peptide	Form.	Site IGF-1R	Cellular IC ₅₀ (M)	Binding IC ₅₀ (M)	Sequence
RP30-IGF	2	1	2.0E-06	1.6E-07	<u>S</u> <u>F</u> <u>S</u> <u>C</u> <u>E</u> <u>S</u> <u>V</u> <u>N</u> <u>G</u> <u>G</u> <u>A</u> <u>E</u> <u>R</u> <u>S</u> <u>D</u> <u>G</u> <u>Q</u> <u>W</u> <u>E</u> <u>G</u> <u>C</u> <u>R</u> SEQ ID NO:2234
RP43	2	1	1.0E-07	1.9E-07	<u>S</u> <u>F</u> <u>S</u> <u>C</u> <u>E</u> <u>S</u> <u>L</u> <u>T</u> <u>G</u> <u>A</u> <u>P</u> <u>Q</u> <u>I</u> <u>R</u> <u>G</u> <u>A</u> <u>W</u> <u>D</u> <u>R</u> <u>C</u> <u>R</u> SEQ ID NO:2235
RP33K-IGF	2	1	2.0E-07	1.8E-09	<u>S</u> <u>F</u> <u>S</u> <u>C</u> <u>E</u> <u>S</u> <u>V</u> <u>N</u> <u>G</u> <u>P</u> <u>A</u> <u>E</u> <u>K</u> <u>S</u> <u>R</u> <u>G</u> <u>Q</u> <u>W</u> <u>D</u> <u>G</u> <u>C</u> <u>R</u> <u>K</u> SEQ ID NO:2266
L-RP9ex	1	1	1.3E-05	2.2E-06	<u>A</u> <u>D</u> <u>Y</u> <u>K</u> <u>D</u> <u>W</u> <u>L</u> <u>D</u> <u>Q</u> <u>E</u> <u>R</u> <u>A</u> <u>W</u> <u>L</u> <u>W</u> <u>C</u> <u>E</u> <u>I</u> <u>S</u> <u>G</u> <u>R</u> <u>G</u> <u>C</u> <u>L</u> <u>S</u> <u>A</u> <u>A</u> <u>G</u> <u>A</u> <u>P</u> <u>A</u> <u>D</u> <u>Y</u> <u>K</u> <u>D</u> <u>D</u> <u>D</u> <u>D</u> <u>K</u> <u>G</u> <u>S</u> <u>L</u> <u>D</u> <u>E</u> <u>S</u> D <u>E</u> <u>R</u> <u>Q</u> <u>L</u> <u>K</u> <u>K</u> <u>A</u> <u>A</u> <u>A</u> <u>G</u> <u>A</u> <u>P</u> <u>V</u> <u>P</u> <u>P</u> <u>D</u> <u>L</u> <u>E</u> <u>P</u> <u>R</u> PG; SEQ ID NO:2231
RP54	Misc.		5.9E-06	4.9E-07	<u>E</u> <u>G</u> <u>S</u> <u>S</u> <u>I</u> <u>C</u> <u>N</u> <u>L</u> <u>L</u> <u>A</u> <u>R</u> <u>A</u> <u>Q</u> <u>I</u> <u>V</u> <u>E</u> <u>L</u> <u>A</u> <u>C</u> <u>E</u> <u>M</u> <u>G</u> <u>V</u> <u>Q</u> <u>E</u> <u>E</u> SEQ ID NO:2246
RP56	Misc.		9.4E-06	6.0E-05	<u>E</u> <u>G</u> <u>Y</u> <u>S</u> <u>W</u> <u>L</u> <u>R</u> <u>D</u> <u>V</u> <u>L</u> <u>M</u> <u>E</u> <u>K</u> <u>Q</u> <u>A</u> <u>Q</u> <u>L</u> <u>K</u> <u>R</u> <u>E</u> <u>G</u> <u>S</u> <u>V</u> <u>G</u> <u>R</u> <u>Q</u> <u>E</u> SEQ ID NO:2247
RP30-IGF-12- RP30-IGF	2-2	1-1	4.6E-06	2.4E-07	<u>A</u> <u>D</u> <u>Y</u> <u>K</u> <u>D</u> <u>S</u> <u>F</u> <u>S</u> <u>C</u> <u>E</u> <u>S</u> <u>V</u> <u>N</u> <u>G</u> <u>G</u> <u>A</u> <u>E</u> <u>R</u> <u>S</u> <u>D</u> <u>G</u> <u>Q</u> <u>W</u> <u>E</u> <u>G</u> <u>C</u> <u>R</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>S</u> S <u>C</u> <u>E</u> <u>S</u> <u>V</u> <u>N</u> <u>G</u> <u>G</u> <u>A</u> <u>E</u> <u>R</u> <u>S</u> <u>D</u> <u>G</u> <u>Q</u> <u>W</u> <u>E</u> <u>G</u> <u>C</u> <u>R</u> <u>A</u> <u>A</u> <u>G</u> A <u>P</u> <u>V</u> <u>P</u> <u>P</u> <u>D</u> <u>L</u> <u>E</u> <u>P</u> <u>R</u> <u>P</u> <u>G</u> ; SEQ ID NO:2241
D8B12-12-RP9	6-1	2-1	4.7E-05	N/A	<u>A</u> <u>D</u> <u>Y</u> <u>K</u> <u>D</u> <u>W</u> <u>L</u> <u>E</u> <u>Q</u> <u>E</u> <u>R</u> <u>A</u> <u>W</u> <u>I</u> <u>W</u> <u>C</u> <u>E</u> <u>K</u> <u>G</u> <u>S</u> <u>G</u> <u>C</u> <u>R</u> <u>A</u> <u>G</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>D</u> <u>D</u> <u>D</u> <u>K</u> <u>G</u> <u>S</u> <u>L</u> <u>D</u> <u>E</u> <u>S</u> D <u>E</u> <u>R</u> <u>Q</u> <u>L</u> <u>K</u> <u>K</u> <u>A</u> <u>A</u> <u>A</u> <u>G</u> <u>A</u> <u>P</u> <u>V</u> <u>P</u> <u>P</u> <u>D</u> <u>L</u> <u>E</u> PRG; SEQ ID NO:2241

Monomer and dimer peptides which block IGF-1 activity in cell proliferation assays in MiaPaCa cells. Form. = formula; Misc. = miscellaneous sequence; Cysteine pairs are shaded and underlined; FY, WF, and L residues from Formula 1 and Formula 2 motifs are shaded and shown in bold.

TABLE 26

IGF-1R Antagonists in FDC-P2 cells

Peptide	Form.	Site IGF-1R	Cellular IC ₅₀ (M)	Binding IC ₅₀ (M)	Sequence
RP30-IGF	2	1	4.0E-06	1.6E-07	SEVSCLESVNGGAERSDQWEGCR SEQ ID NO:2234
RP9-lig	1	1	3.2E-04	2.2E-07	GSLDESVDWFERQLGKK-Lig SEQ ID NO:
lig-RP9	1	1	8.8E-08	1.5E-06	Lig-GSLDESVDWFERQLGKK SEQ ID NO:
RP43	2	1	7.0E-06	1.9E-07	SEVSCLESVNGGALTGAPQPIRGAWDRCR SEQ ID NO:2235
H2C-A-H6	1	1	3.0E-07	3.2E-05	VGRASGFPEVDWFERQLSLQSGEQ SEQ ID NO:2228
RP6KK	2	1	1.0E-06	N/A	TEVSCLESVNGGALTGTPQPNRGPWERCCKK SEQ ID NO:2236
C1	1	1	4.0E-06	4.2E-08	DYKDCWARPCGDAANVDWFERQLGKK SEQ ID NO:2230
RP33-IGF	2	1	7.0E-06	1.9E-06	SEVSCLESVNGGPAKSRGQWDGCR SEQ ID NO:2232
RP6	2	1	5.0E-06	3.5E-07	TEVSCLESVNGGALTGTPQPNRGPWERC SEQ ID NO:2236
RP9	1	1	2.0E-06	9.7E-07	GSLDESVDWFERQLGKK SEQ ID NO:2229
RP9-RP9 (C-C)	1-1	1-1	3.0E-05	1.2E-07	(GSLDESVDWFERQLGKK) ₂ -17 SEQ ID NO:2237
RP9-RP9 (C-N)	1-1	1-1	3.0E-05	1.7E-07	GSLDESVDWFERQLGKK-Lig)-19-(Lig-GSLDESVDWFERQLGKK) SEQ ID NO:2238
G33-RP9	1-1	1-1	1.0E-06	N/A	ADYKDGIIISQSPESVDWFERQVSDPWWCWGSLDESVDWFERQLAAAGAPVPDPLEPRPG SEQ ID NO:2240

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Example 15: IGF-1 Agonist Peptides**A. MCF-7 and MiaPaCa Cell Assays**

Peptides produced synthetically were maintained as 30 mM stock in 100% DMSO, while recombinant dimers were diluted in water. All synthetic and recombinant peptides were stored at -80°C. The final concentration of DMSO was < 0.1%. MCF-7 and MiaPaCa (ATCC, Rockville, MD) cells were maintained in RPMI containing 10% FBS. All cells were starved overnight by growing them in serum-free RPMI media. Cells were trypsinized, washed twice with PBS before being seeded at $1-3 \times 10^3$ cells per well in a 96-well plate in a volume of 150 μ l/well. All points were done in duplicate in 96-well plates. For agonist activity assays, immediately before the addition of peptides, all media was gently removed from the wells. Peptides were serially diluted 1:2 in a final volume of 150 μ l in a separate plate using RPMI containing 0.1% FBS. The diluted peptide solutions were transferred onto the cells, and the plates were incubated for 72 h at 37°C in a CO₂ incubator. To quantitate cell number, 10 μ l of WST-1 reagent (Roche Molecular Biochemicals, Indianapolis, IN) was added to each well and the plates were returned to the 37°C/CO₂ incubator for approximately 2 h. Measurements were then taken at 440 nm, with 700 nm used as a reference.

B. FDC-P2 Cell Assays

Peptides were maintained and stored as indicated above. FDC-P2 cells (obtained from Dr. J. Pierce, NIH) were maintained in RPMI containing 15% FBS and 5% WEHI (Genoquest, Germantown, MD) at 37°C in a CO₂ incubator. To initiate experiments, all cells were starved for 5 h in RPMI containing 1% FBS. Cells were seeded at 1×10^4 cells per well into 96-well plates at a volume of 75 μ l/well. Peptides were added at 2X final concentration and all points were done in duplicate. For agonist assays, peptides at 2X concentration were serially diluted 1:2 in a final volume of 75

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μl in a separate plate using RPMI containing 0.1% FBS. The diluted peptide solutions were transferred onto the cells to yield a final volume of 150 μl. The plates were incubated for 48 h at 37°C in a CO₂ incubator. To quantitate cell number, 10 μl of WST-1 reagent (Roche Molecular
5 Biochemicals, Indianapolis, IN) was added to each well and the plates were returned to the 37°C incubator for approximately 2 h. Measurements were taken at 440 nm, with 700 nm used as a reference.

For these experiments, potencies of peptide competition were determined using the AlphaScreen assay format. Primary data were
10 background corrected, normalized to buffer controls and then expressed as % specific binding. The data were fit to a four-parameter non-linear regression analysis ($y = \min + (\max - \min) / (1 + 10^{((\log IC_{50} - x) * \text{Hillslope}))}$), which was used to determine IC₅₀ values. The Z'-factor for this assay is greater than 0.7 ($Z' = 1 - (3\sigma_+ + 3\sigma_-) / |\mu_+ - \mu_-|$) and the signal-to-background (S/B)
15 ratio was between 40 and 70.

C. Results

Several IGF-1R agonist peptides were identified which consistently stimulated proliferation of both MCF-7 and MiaPaCa cells (Tables 27-28; Figures 73A-73D and Figures 74A-74I). Monomer peptides with IGF-1R
20 agonist activity included RP60, RP48, G33, C1, and L-RP9ex (Tables 27-28). Dimer peptides with IGF-1R agonist activity included RP30-IGF-12-D112, RP30-IGF-12-RP31-IGF, RP31-IGF-12-RP30-IGF, D112-12-RP30-IGF, RP6-L-D8B12, D8B12-12-RP9, D112-12-D112, RP9-12-RP9, and RP9-L-RP6 (Tables 27-28). Agonist peptides were also identified using
25 FDC-P2 cell proliferation assays (Table 29). Monomer peptides with IGF-1R agonist activity included G33-lig, G33, S175, D815, lig-D815, RP31-IGF, and D815 (Table 29). Dimer peptides with IGF-1R agonist activity included RP6-RP9, G33-6-G33, and D815-RP9 (Table 29).

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In addition, peptides with agonist or antagonist activity in MCF-7 or MiaPaCa cell proliferation assays were shown to compete against IGF-1 for binding to IGF-1R (Figures 71A-71F and Figures 72A-72E). Potencies of peptide competition were determined using the AlphaScreen assay format
5 for peptide monomers RP60, RP48, sG33, L-RP9ex, and 12-RP9ex (Figures 71A-71F). Potencies were also determined for dimer peptides rRP30-IGF-12-D112, rRP30-IGF-12-RP31-IGF, rRP31-IGF-12-RP30-IGF, rD112-12-RP30-IGF, and rD112-12-D112 (Figures 72A-72E).

The biological response of the monomers and dimers in the FDC-P2
10 (myeloid cells; IGF-1R/IGF-1R receptor), MCF-7 (breast cancer cells; hybrid IGF-1R/IR receptor) and MiaPaCa (pancreatic cancer cells; hybrid IGF-1R/IR receptor) assays were compared (Table 30). In some instances, a modulatory effect (agonism or antagonism) was seen in certain cell lines but not in others. For example, the RP30-IGF peptide exhibited antagonist
15 activity in FDC-P2 and MiaPaCa cells, but not in MCF-7 cells (Table 30). The C1 peptide exhibited antagonist activity in FDC-P2 and MCF-7 cells, but not in MiaPaCa cells. The RP9-RP6, L-RP9ex, and D8B12-12-RP9 peptides exhibited either antagonist or agonist activity depending on the cell line used (Table 30). Therefore, it is possible to use the peptides of the
20 invention to target specific cell types with specific modulatory effects.

TABLE 27

IGF-1R Agonists in MCF-7 Cells

Peptide	Form.	Site IGF-1R	Cellular EC ₅₀ (M)	Binding IC ₅₀ (M)	Sequence
G33	1	1	3.0E-07	6.0E-07	GIISQSCPES ₁ YD ₂ W ₃ FAGQVSDPWWQW SEQ ID NO:2249
12-RP9ex	1	1	1.0E-05	4.5E-07	ISGRGCLSGSGSGSGSGSLDES ₁ YD ₂ W ₃ ERQLGKAAAGAPVPYDPDPLEPRPG SEQ ID NO:2250
L-RP9ex	1	1	4.2E-06	2.2E-06	ADYKD ₁ WLDQGERAWLWCEISGRGCLSAAGAPADYKDDDDKGSLSDES ₁ YD ₂ W ₃ ERQLKAAAGAPVPYDPDPLEPRPG G; SEQ ID NO:2231
RP48	Misc.		3.2E-06	6.6E-07	EDWLRCLGVILSGGLTELANTGCVQGE SEQ ID NO:2261
RP60	Misc.		2.1E-06	1.6E-05	ELQGFCELLATVTGVTGLGLDYQPIE SEQ ID NO:2262
RP9-12-RP9	1-1	1-1	2.4E-06	N/A	ADYKDDDDKGSLSDES ₁ YD ₂ W ₃ ERQLGKK GSGSGSGSGSGS DDDKGSLSDES ₁ YD ₂ W ₃ ERQLGKK AAAGAPVPYDPDPLEPRG; SEQ ID NO:2254
RP9-L-RP6	1-2	1-1	1.3E-06	N/A	ADYKDDDDKGSLSDES ₁ YD ₂ W ₃ ERQLAAAGAPADYKDT ₁ SC ₂ AS ₃ LTGTPQPNRGPWERCRAAAGAPVPYDPDPLE PRPG; SEQ ID NO:2242
D112-12-RP30-IGF	1-2	1-1	2.5E-07	1.7E-07	ADYKDCQWARP ₁ CGDAAN ₂ YD ₃ W ₄ VQQASKKGGSGSGSGSGSS ₅ SC ₆ ES ₇ VNGGAERSDGQWEGCRAAAGAP VPYDPDPLEPRPG; SEQ ID NO:2257
D112-12-D112	1-1	1-1	3.5E-07	6.2E-07	ADYKDCQWARP ₁ CGDAAN ₂ YD ₃ W ₄ VQQASKKGGSGSGSGSGSGSWARP ₅ CGDAAN ₆ YD ₇ W ₈ VQQASKKAAAGAPVP YPDPLEPRPG; SEQ ID NO:2255
RP30-IGF-12-D112	2-1	1-1	4.1E-07	1.1E-06	ADYKDS ₁ YSC ₂ ES ₃ VNGGAERSDGQWEGC ₄ RGSGSGSGSGSGSWARP ₅ CGDAAN ₆ YD ₇ W ₈ VQQASKKAAAGAP VPYDPDPLEPRPG; SEQ ID NO:2258
RP6-L-D8B12	2-6	1-2	7.1E-07	N/A	ADYKDT ₁ YSC ₂ AS ₃ LTGTPQPNRGPWERCRAAAGAPADYKDWLEQERAWIWEKGGCRAAAGAPVPYDPDPLE PRPG; SEQ ID NO:2263
RP30-IGF-12-RP31-IGF	2-6	1-2	1.1E-07	1.1E-06	ADYKDS ₁ YSC ₂ ES ₃ VNGGAERSDGQWEGC ₄ RGSGSGSGSGSGVDEVRAWLW ₅ CQISGLG ₆ CESK ₇ AAAGAPVP YPDPLEPRPG; SEQ ID NO:2264
RP31-IGF-12-RP30-IGF	6-2	2-1	3.0E-06	7.2E-08	ADYKDG ₁ VDEVRAWLW ₂ CQISGLG ₃ ESK ₄ GGSGSGSGSGSS ₅ SC ₆ ES ₇ VNGGAERSDGQWEGCRAAAGAPVP YPDPLEPRPG; SEQ ID NO:2265
D8B12-12-RP9	6-1	2-1	3.5E-07	N/A	ADYKDWLEQERAWIWEK ₁ GGC ₂ RAGSGSGSGSGSDDDKGSLSDES ₃ YD ₄ W ₅ ERQLGKAAAGAPVPYDPDPLE PRG; SEQ ID NO:2241

Monomer and dimer peptides which stimulate cell proliferation using the MCF-7 cells. Form. = formula; N/A = not available; Misc. = miscellaneous sequence ; Cysteine pairs are shaded and underlined; FY, WF, and L residues from Formula 1 and Formula 2 motifs are shaded and shown in bold. .

TABLE 28

IGF-1R Agonists in MiaPaCa Cells

Peptide	Form.	Site	Cellular EC50 (M)	Binding IC50 (M)	Sequence
RP48	Misc.		7.0E-06	6.6E-07	EDWLRCLGVLSGGLTELANTGCVQGE SEQ ID NO:2261
RP60	Misc.		3.1E-06	1.6E-05	ELQGFCELLATVTGVTGLGCLDYQPIE SEQ ID NO:2262
RP9-L-RP6	1-2	1-1	1.0E-06	N/A	ADYKDDDDKGSIDES DW WF ERQLAAAGAPADYKDT SC AS LTGTPQPNRGPWERCRAAAGAPVPYDPDPLE PRPG; SEQ ID NO:2242
D112-12-RP30-IGF	1-2	1-1	2.2E-06	1.7E-07	ADYKDCWARP CGDAAN DW WF VQQAASKKGGSGGGSGSS SC ES VNGGAERSDGGQWEGCRAAAGAP VPYDPLEPRPG; SEQ ID NO:2257
D112-12-D112	1-1	1-1	2.5E-06	6.2E-07	ADYKDCWARP CGDAAN DW WF VQQAASKKGGSGGGSGSGSWARP CGDAAN D W VQQAASKKAAAGAPVP YPDPLEPRPG; SEQ ID NO:2255
RP9-12-RP9	1-1	1-1	2.7E-06	N/A	ADYKDDDDKGSIDES DW WF ERQLGKK GGSGGGSGSGS DDD KGSLDES D W ERQLGKK AAAGAPVPYDPDPLEPRPG; SEQ ID NO:2254
RP6-L-D8B12	2-6	1-2	5.0E-06	N/A	ADYKDT SC AS LTGTPQPNRGPWERCRAAAGAPADYKDWLEQERAWIWCEKGGCRAAAGAPVPYDPDPLE PRPG; SEQ ID NO:2263
RP31-IGF-12-RP30-IGF	6-2	2-1	4.4E-06	7.2E-08	ADYKDGDEVRAWLWCQISGLGCESKGGSGGGSGSS SC ES VNGGAERSDGGQWEGCRAAAGAPVP YPDPLEPRPG; SEQ ID NO:2265
RP30-IGF-12-RP31-IGF	2-6	1-2	6.8E-05	1.1E-06	ADYKDS SC ES VNGGAERSDGGQWEGCRGGSGGGSGSGVDEVRAWLWCQISGLGCESKAAAGAPVP YPDPLEPRPG; SEQ ID NO:2264
D8B12-12-RP9	6-1	2-1	1.0E-06	N/A	ADYKDWLEQERAWIWCEKGGCRAGGGSGGGSGGSDDDKGSIDES DW WF ERQLGKKAAAGAPVPYDPDPLE PRPG; SEQ ID NO:2241

Monomer and dimer peptides which stimulate cell proliferation using the MiaPaCa cells. Form. = formula; N/A = not available; Misc. = miscellaneous sequence; Cysteine pairs are shaded and underlined; FY, WF, and L residues from Formula 1 and Formula 2 motifs are shaded and shown in bold.

TABLE 29

IGF-1R Agonists in FDC-P2 Cells

Peptide	Form.	Site IGF-1R	Cellular EC ₅₀ (M)	Binding IC ₅₀ (M)	Sequence
G33-lig	1	1	3.0E-06	7.1E-07	GIISQSCPES DW AGQVSDPWWC <u>W</u> Lig SEQ ID NO:
G33	1	1	2.0E-06	6.0E-07	GIISQSCPES DW AGQVSDPWWC <u>W</u> SEQ ID NO:2249
S175	1	1	1.0E-05	7.1E-06	GRVDWLQRNAN DW VAELG SEQ ID NO:2248
D815	6	2	3.0E-06	1.1E-05	WLDQERAWLWCEISGRG <u>C</u> LS SEQ ID NO:2252
lig-D815	6	2	1.0E-05	2.2E-06	Lig-WLDQERAWLWCEISGRG <u>C</u> LS SEQ ID NO:
RP31-IGF	6	2	2.0E-05	8.4E-07	GVDEVRAWLW <u>C</u> QISGLG <u>C</u> ES SEQ ID NO:2253
D815	6	2	1.0E-06	1.5E-06	WLDQERAWLWCEISGRG <u>C</u> LS SEQ ID NO:2252
RP6-RP9	2-1	1-1	6.0E-06	N/A	ADYKDT DW SC <u>W</u> AS DW LTGTPQPNRGPWERC <u>G</u> SLDES DW ERQLAAAGAPVPYDPLEPRPG SEQ ID NO:2259
G33-6-G33	1-1	1-1	3.0E-06	1.4E-07	ADYKDGISQSCPES DW AGQVSDPWWC <u>W</u> GGGGISQSCPES DW AGQVSDPWWC <u>W</u> KKAAAGAPVPYDPLEPRPG; SEQ ID NO:2256
D815-RP9	6-1	2-1	3.0E-06	1.1E-06	WLDQERAWLWCEISGRG <u>C</u> LSGLDES DW ERQLG SEQ ID NO:2260

Monomer and dimer peptides which stimulate cell proliferation using the FDC-P2 cells. Form. = formula; N/A = not available; Lig = Diaminopropionic acid with a 2-aminohydroxyacetyl group (CO-CH₂-O-NH₂) on the side chain amino group; Cysteine pairs are shaded and underlined; FY, WF, and L residues from Formula 1 and Formula 2 motifs are shaded and shown in bold.

TABLE 30

Peptide	Site IGF/Formula	FDC-P2 Agonist	FDC-P2 Antagonist	MCF-7 Agonist	MCF-7 Antagonist	MiaPaCa Agonist	MiaPaCa Antagonist
Monomers:							
D815	2 (cys.) 6	+					
RP30-IGF	1 (cys.) 2		+	No	No		+
RP31-IGF	2 (cys.) 6	+		+		No	No
G33	1 (cys.) 1	+		+		+	
RP9	1 1		+	No	No	No	No
RP6	1 (cys.) 2		+		+	No	No
C1	1 (cys.) 1		+		+	No	No
RP33-IGF	1 (cys.) 2		+		+		+
H2C-A-H6	1 1	ND	ND		+		+
RP43	1 (cys.) 2		+	No	No		+
RP48	Misc. (cys.)	ND	ND	+		+	
RP52	Misc. (cys.)	ND	ND	No	No		+
RP54	Misc. (cys.)	ND	ND		+		+
RP56	Misc.	ND	ND	No	No		+
RP60	Misc. (cys.)	ND	ND	+		+	
Dimers:							
RP9-12-RP9	1-1 1-1	ND	ND	+		+	
D8B12-12-RP9	2-1 (cys.) 2-1	ND	ND	+		+	
RP9-L-RP6	1-1 (cys.) 1-2		+	+		+	
RP6-D8B12	1-2 (cys.) 2-2	ND	ND	+		+	

RP30-IGF-C1	1-1 (cys.) 2-1	ND	ND	+		+	
C1-RP30-IGF	1-1 (cys.) 1-1	ND	ND	+		+	
RP30-IGF-12-RP30-IGF	1-1 (cys.) 2-2	ND	ND		+		+
RP30-IGF-12-RP31-IGF	1-2 (cys.) 2-2	ND	ND	+		+	
RP31-IGF-12-RP30-IGF	2-1 (cys.) 2-2	ND	ND	+		+	
RP6-D8B12	1-2 (cys.) 2-2	ND	ND	+		+	

ND =Not Done; + = Effect observed; No = No effect observed; Cys. = contains putative cysteine loop.

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Incorporated herein by reference in its entirety is the Sequence Listing for the application, comprising SEQ ID NO:1 to SEQ ID NO:2227. The Sequence Listing is disclosed on three CD-ROMs, designated "CRF", "Copy 1", and "Copy 2". The Sequence Listing is a computer-readable
5 ASCII file named "18784056PC.app.txt", created on September 23, 2002, in IBM-PC machine format, on a MS-Windows®98 operating system. The 18784056PC.app.txt file is 927,477 bytes in size.

As various changes can be made in the above compositions and
10 methods without departing from the scope and spirit of the invention, it is intended that all subject matter contained in the above description, shown in the accompanying drawings, or defined in the appended claims be interpreted as illustrative, and not in a limiting sense.

15 The contents of all patents, patent applications, published articles, books, reference manuals, texts and abstracts cited herein are hereby incorporated by reference in their entirety to more fully describe the state of the art to which the present invention pertains.

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WHAT IS CLAIMED IS:

1. A method of modulating insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to modulate the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 1 sequence, $X_1X_2X_3X_4X_5$, such that X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine; and ii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.
2. The method of claim 1, wherein the amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.
3. The method of claim 2, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:
 - a. H2C-A-H6 (SEQ ID NO:2228); and
 - b. RP9 (SEQ ID NO:2229).
4. The method of claim 2, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:
 - a. S175 (SEQ ID NO:2248); and
 - b. 12-RP9ex (SEQ ID NO:2250).

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5. The method of claim 2, wherein the amino acid sequence further comprises two or more cysteines which are separated by at least 3 amino acids.
6. The method of claim 5, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:
 - a. C1 (SEQ ID NO:2230);
 - b. C1KK (SEQ ID NO:2266); and
 - c. L-RP9ex (SEQ ID NO:2231).
7. The method of claim 5, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:
 - a. G33 (SEQ ID NO:2249); and
 - b. L-RP9ex (SEQ ID NO:2231).
8. A method of decreasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to decrease the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 2 sequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} , and X_{12} are any amino acid, and X_{10} and X_{13} are leucine; ii) the amino acid sequence further comprises two or more cysteines which are separated by at least 3 amino acids; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

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9. The method of claim 8, wherein the amino acid sequence decreases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

10. The method of claim 9, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

- a. RP33-IGF (SEQ ID NO:2232);
- b. RP6KK (SEQ ID NO:2233);
- c. RP30-IGF (SEQ ID NO:2234);
- d. RP43 (SEQ ID NO:2235);
- e. RP33K-IGF (SEQ ID NO:2266); and
- f. RP6 (SEQ ID NO:2236).

11. A method of increasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to increase the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 6 sequence, $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, such that X_{62} , X_{65} , X_{66} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid, X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine, X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine, and methionine, X_{64} is selected from group consisting of aspartic acid and glutamic acid, X_{67} is tryptophan, X_{75} is selected from group consisting of tyrosine and tryptophan, and X_{72} and X_{79} are cysteines; and ii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

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12. The method of claim 11, wherein the amino acid sequence increases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

13. The method of claim 12, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

- a. D815 (SEQ ID NO:2252); and
- b. RP31-IGF (SEQ ID NO:2253).

14. A method of modulating insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to modulate the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises at least two Formula 1 subsequences, $X_1X_2X_3X_4X_5$, such that X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine; and ii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

15. The method of claim 14, wherein the amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

16. The method of claim 15, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

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- a. RP9-RP9 (C-C; SEQ ID NO:2237);
- b. RP9-RP9 (C-N; SEQ ID NO:2238); and
- c. RP9-L-RP9 (SEQ ID NO:2239).

17. The method of claim 15, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence, RP9-12-RP9 (SEQ ID NO:2254).

18. The method of claim 15, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids.

19. The method of claim 18, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, G33-RP9 (SEQ ID NO:2240).

20. The method of claim 18, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

- a. D112-12-D112 (SEQ ID NO:2255); and
- b. G33-6-G33 (SEQ ID NO:2256).

21. A method of decreasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to decrease the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises at least two Formula 2 subsequences, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} , and X_{12} are any amino acid, and X_{10} and X_{13} are leucine; ii) the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

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22. The method of claim 21, wherein the amino acid sequence decreases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.
23. The method of claim 22, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, RP30-IGF-12-RP30-IGF (SEQ ID NO:2241).
24. A method of modulating insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to modulate the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 2 subsequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} , and X_{12} are any amino acid, and X_{10} and X_{13} are leucine; ii) the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; iii) the subsequences are oriented Formula 1 to Formula 2; and iv) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.
25. The method of claim 24, wherein the amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

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26. The method of claim 25, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, RP9-L-RP6 (SEQ ID NO:2242).

27. The method of claim 25, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

- a. RP9-L-RP6 (SEQ ID NO:2242); and
- b. D112-12-RP30-IGF (SEQ ID NO:2257).

28. A method of increasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to increase the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 2 subsequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} , and X_{12} are any amino acid, and X_{10} and X_{13} are leucine; ii) the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; iii) the subsequences are oriented Formula 2 to Formula 1; and iv) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

29. The method of claim 28, wherein the amino acid sequence increases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

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30. The method of claim 29, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

- a. RP30-IGF-12-D112 (SEQ ID NO:2258); and
- b. RP6-RP9 (SEQ ID NO:2259).

31. A method of decreasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to decrease the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 6 subsequence, $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, such that X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_{62} , X_{65} , X_{66} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid, X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; X_{75} is selected from group consisting of tyrosine and tryptophan, and X_{72} and X_{79} are cysteines; ii) the subsequences are oriented Formula 1 to Formula 6; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

32. The method of claim 31, wherein the amino acid sequence decreases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

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33. The method of claim 32, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, G33-D8B12 (SEQ ID NO:2243).

34. A method of modulating insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to modulate the activity of insulin-like growth factor receptor, wherein i) the amino acid sequence comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 6 subsequence, $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, such that X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_{62} , X_{65} , X_{66} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid, X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; X_{75} is selected from group consisting of tyrosine and tryptophan, and X_{72} and X_{79} are cysteines; ii) the subsequences are oriented Formula 6 to Formula 1; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

35. The method of claim 34, wherein the amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

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36. The method of claim 35, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, D8B12-12-RP9 (SEQ ID NO:2244).

37. The method of claim 35, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

- a. D8B12-12-RP9 (SEQ ID NO:2244); and
- b. D815-RP9 (SEQ ID NO:2260).

38. A method of decreasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to decrease the activity of insulin-like growth factor receptor, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

- a. H2C-A-H6 (SEQ ID NO:2228);
- b. RP9 (SEQ ID NO:2229);
- c. C1 (SEQ ID NO:2230);
- d. L-RP9ex (SEQ ID NO:2231);
- e. RP33-IGF (SEQ ID NO:2232);
- f. RP6KK (SEQ ID NO:2233);
- g. RP30-IGF (SEQ ID NO:2234);
- h. RP43 (SEQ ID NO:2235);
- i. RP6 (SEQ ID NO:2236);
- j. RP52 (SEQ ID NO:2245);
- k. RP54 (SEQ ID NO:2246);

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- l. RP33K-IGF (SEQ ID NO:2266);
- m. C1KK (SEQ ID NO:2266); and
- n. RP56 (SEQ ID NO:2247).

39. A method of decreasing insulin-like growth factor receptor activity in activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to decrease the activity of insulin-like growth factor receptor, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

- a. RP9-RP9 (C-C; SEQ ID NO:2237);
- b. RP9-RP9 (C-N; SEQ ID NO:2238);
- c. RP9-L-RP9 (SEQ ID NO:2239);
- d. G33-RP9 (SEQ ID NO:2240);
- e. RP30-IGF-12-RP30-IGF (SEQ ID NO:2241);
- f. RP9-L-RP6 (SEQ ID NO:2242);
- g. G33-D8B12 (SEQ ID NO:2243); and
- h. D8B12-12-RP9 (SEQ ID NO:2244).

40. A method of increasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to increase the activity of insulin-like growth factor receptor, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

- a. S175 (SEQ ID NO:2248);
- b. G33 (SEQ ID NO:2249);
- c. 12-RP9ex (SEQ ID NO:2250);

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- d. L-RP9ex (SEQ ID NO:2231);
- e. D815 (SEQ ID NO:2252);
- f. RP31-IGF (SEQ ID NO:2253);
- g. RP48 (SEQ ID NO:2261); and
- h. RP60 (SEQ ID NO:2262).

41. A method of increasing insulin-like growth factor receptor activity in insulin-like growth factor-responsive mammalian cells comprising: contacting the cells with an amino acid sequence in an amount sufficient to increase the activity of insulin-like growth factor receptor, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

- a. RP9-12-RP9 (SEQ ID NO:2254);
- b. D112-12-D112 (SEQ ID NO:2255);
- c. G33-6-G33 (SEQ ID NO:2256);
- d. RP9-L-RP6 (SEQ ID NO:2242);
- e. D112-12-RP30-IGF (SEQ ID NO:2257);
- f. RP9-L-RP6 (SEQ ID NO:2242);
- g. RP30-IGF-12-D112 (SEQ ID NO:2258);
- h. RP6-RP9 (SEQ ID NO:2259);
- i. D8B12-12-RP9 (SEQ ID NO:2244);
- j. RP6-L-D8B12 (SEQ ID NO:2263);
- k. RP30-IGF-12-RP31-IGF (SEQ ID NO:2264);
- l. RP31-IGF-12-RP30-IGF (SEQ ID NO:2265); and
- m. D815-RP9 (SEQ ID NO:2260).

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42. An insulin-like growth factor receptor modulator comprising an amino acid sequence which comprises a Formula 1 sequence, $X_1X_2X_3X_4X_5$, such that X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; and with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

43. The insulin-like growth factor receptor modulator of claim 42, wherein amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

44. The insulin-like growth factor receptor modulator of claim 43, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence selected from the group consisting of:

- a. C1 (SEQ ID NO:2230);
- b. C1KK (SEQ ID NO:2266); and
- c. L-RP9ex (SEQ ID NO:2231).

45. The insulin-like growth factor receptor modulator of claim 43, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

- a. G33 (SEQ ID NO:2249); and
- b. L-RP9ex (SEQ ID NO:2231).

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46. An insulin-like growth factor receptor antagonist comprising an amino acid sequence which comprises a Formula 2 sequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} , and X_{12} are any amino acid, and X_{10} and X_{13} are leucine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

47. The insulin-like growth factor receptor antagonist of claim 46, wherein amino acid sequence decreases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

48. The insulin-like growth factor receptor antagonist of claim 47, wherein the amino acid sequence comprises a sequence selected from the group consisting of:

- a. RP33-IGF (SEQ ID NO:2232);
- b. RP6KK (SEQ ID NO:2233);
- c. RP30-IGF (SEQ ID NO:2234);
- d. RP43 (SEQ ID NO:2235);
- e. RP33K-IGF (SEQ ID NO:2266); and
- f. RP6 (SEQ ID NO:2236).

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49. An insulin-like growth factor receptor modulator comprising an amino acid sequence which comprises at least two Formula 1 subsequences, $X_1X_2X_3X_4X_5$, such that X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

50. The insulin-like growth factor receptor modulator of claim 49, wherein amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

51. The insulin-like growth factor receptor modulator of claim 50, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, G33-RP9 (SEQ ID NO:2240).

52. The insulin-like growth factor receptor modulator of claim 50, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

- a. D112-12-D112 (SEQ ID NO:2255); and
- b. G33-6-G33 (SEQ ID NO:2256).

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53. An insulin-like growth factor receptor antagonist comprising an amino acid sequence that comprises at least two Formula 2 subsequences, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} , and X_{12} are any amino acid, and X_{10} and X_{13} are leucine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

54. The insulin-like growth factor receptor antagonist of claim 53, wherein amino acid sequence decreases insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

55. The insulin-like growth factor receptor antagonist of claim 54, wherein amino acid sequence comprises the sequence RP30-IGF-12-RP30-IGF (SEQ ID NO:2241).

56. An insulin-like growth factor receptor modulator comprising an amino acid sequence which comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 2 subsequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} , and X_{12} are any amino acid, and X_{10} and X_{13} are leucine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids, and wherein the subsequences are oriented Formula 1 to Formula 2, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

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57. The insulin-like growth factor receptor modulator of claim 56, wherein amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

58. The insulin-like growth factor receptor modulator of claim 57, wherein the amino acid sequence comprises an insulin-like growth factor receptor antagonist sequence, RP9-L-RP6 (SEQ ID NO:2242).

59. The insulin-like growth factor receptor modulator of claim 57, wherein the amino acid sequence comprises an insulin-like growth factor receptor agonist sequence selected from the group consisting of:

- a. RP9-L-RP6 (SEQ ID NO:2242); and
- b. D112-12-RP30-IGF (SEQ ID NO:2257).

60. An insulin-like growth factor receptor agonist comprising an amino acid sequence which comprises a Formula 1 subsequence $X_1X_2X_3X_4X_5$, and a Formula 2 subsequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine, and such that X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} , and X_{12} are any amino acid, and X_{10} and X_{13} are leucine, wherein the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids, and wherein the subsequences are oriented Formula 2 to Formula 1, with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof.

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61. The insulin-like growth factor receptor agonist of claim 60, wherein amino acid sequence modulates insulin-like growth factor receptor activity in mammalian cells selected from the group consisting of breast cancer cells, pancreatic cancer cells, and myeloid cells.

62. The insulin-like growth factor receptor agonist of claim 61, wherein the amino acid sequence comprises a sequence selected from the group consisting of:

- a. RP30-IGF-12-D112 (SEQ ID NO:2258); and
- b. RP6-RP9 (SEQ ID NO:2259).

63. An insulin-like growth factor receptor antagonist comprising an amino acid sequence selected from the group consisting of:

- a. H2C-A-H6 (SEQ ID NO:2228);
- b. L-RP9ex (SEQ ID NO:2231);
- c. RP33-IGF (SEQ ID NO:2232);
- d. RP30-IGF (SEQ ID NO:2234);
- e. RP43 (SEQ ID NO:2235);
- f. G33-RP9 (SEQ ID NO:2240);
- g. RP30-IGF-12-RP30-IGF (SEQ ID NO:2241);
- h. RP9-L-RP6 (SEQ ID NO:2242);
- i. G33-D8B12 (SEQ ID NO:2243);
- j. D8B12-12-RP9 (SEQ ID NO:2244);
- k. RP52 (SEQ ID NO:2245);
- l. RP54 (SEQ ID NO:2246);
- m. RP33K-IGF (SEQ ID NO:2266); and
- n. RP56 (SEQ ID NO:2247).

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64. An insulin-like growth factor receptor agonist comprising an amino acid sequence selected from the group consisting of:

- a. 12-RP9ex (SEQ ID NO:2250);
- b. L-RP9ex (SEQ ID NO:2231);
- c. RP31-IGF (SEQ ID NO:2253);
- d. D112-12-D112 (SEQ ID NO:2255);
- e. G33-6-G33 (SEQ ID NO:2256);
- f. RP9-L-RP6 (SEQ ID NO:2242);
- g. D112-12-RP30-IGF (SEQ ID NO:2257);
- h. RP30-IGF-12-D112 (SEQ ID NO:2258);
- i. RP6-RP9 (SEQ ID NO:2259);
- j. D8B12-12-RP9 (SEQ ID NO:2244);
- k. D815-RP9 (SEQ ID NO:2260);
- l. RP48 (SEQ ID NO:2261); and
- m. RP60 (SEQ ID NO:2262).

65. A method of identifying an insulin-like growth factor receptor modulator comprising:

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a. contacting insulin-like growth factor receptor with an amino acid sequence to form a complex, wherein i) the amino acid sequence comprises a Formula 1 sequence, $X_1X_2X_3X_4X_5$, such that X_1 , X_2 , and X_5 are selected from the group consisting of phenylalanine and tyrosine, X_3 is selected from the group consisting of aspartic acid, glutamic acid, glycine and serine, and X_4 is selected from group consisting of tryptophan, tyrosine and phenylalanine; ii) the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof;

b. contacting the complex of (a) with a compound library;

c. identifying a compound which disrupts the complex of (a); and

d. determining whether the compound exhibits agonist or antagonist activity at insulin-like growth factor receptor, wherein this activity indicates identification of an insulin-like growth factor receptor modulator.

66. The method of claim 65, wherein the amino acid sequence comprises a sequence selected from the group consisting of:

a. C1 (SEQ ID NO:2230);

b. L-RP9ex (SEQ ID NO:2231);

c. G33 (SEQ ID NO:2249);

d. C1KK (SEQ ID NO:2266); and

e. L-RP9ex (SEQ ID NO:2231).

67. A method of identifying an insulin-like growth factor receptor modulator comprising:

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a. contacting insulin-like growth factor receptor with an amino acid sequence to form a complex, wherein i) the amino acid sequence comprises a Formula 2 sequence, $X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}$, such that X_6 and X_7 are aromatic amino acids, X_8 , X_9 , X_{11} , and X_{12} are any amino acid, and X_{10} and X_{13} are leucine; ii) the amino acid sequence further comprises two or more cysteines separated by at least 3 amino acids; and iii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof;

b. contacting the complex of (a) with a compound library;

c. identifying a compound which disrupts the complex of (a); and

d. determining whether the compound exhibits agonist or antagonist activity at insulin-like growth factor receptor, wherein this activity indicates identification of an insulin-like growth factor receptor modulator.

68. The method of claim 67, wherein the amino acid sequence comprises a sequence selected from the group consisting of:

a. RP33-IGF (SEQ ID NO:2232);

b. RP6KK (SEQ ID NO:2233);

c. RP30-IGF (SEQ ID NO:2234);

d. RP43 (SEQ ID NO:2235);

e. RP33K-IGF (SEQ ID NO:2266); and

f. RP6 (SEQ ID NO:2236).

69. A method of identifying an insulin-like growth factor receptor modulator comprising:

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a. contacting insulin-like growth factor receptor with an amino acid sequence to form a complex, wherein i) the amino acid sequence comprises a Formula 6 sequence, $X_{62} X_{63} X_{64} X_{65} X_{66} X_{67} X_{68} X_{69} X_{70} X_{71} X_{72} X_{73} X_{74} X_{75} X_{76} X_{77} X_{78} X_{79} X_{80} X_{81}$, such that X_{62} , X_{65} , X_{66} , X_{68} , X_{69} , X_{71} , X_{73} , X_{76} , X_{77} , X_{78} , X_{80} and X_{81} are any amino acid, X_{63} is selected from the group consisting of leucine, isoleucine, methionine and valine; X_{70} and X_{74} are selected from group consisting of valine, isoleucine, leucine and methionine; X_{64} is selected from group consisting of aspartic acid and glutamic acid; X_{67} is tryptophan; X_{75} is selected from group consisting of tyrosine and tryptophan, and X_{72} and X_{79} are cysteines; and ii) with the proviso that the amino acid sequence is not insulin, insulin-like growth factor, an anti-insulin receptor antibody, an anti-insulin-like growth factor receptor antibody, or fragments thereof;

b. contacting the complex of (a) with a compound library;

c. identifying a compound which disrupts the complex of (a); and

d. determining whether the compound exhibits agonist or antagonist activity at insulin-like growth factor receptor, wherein this activity indicates identification of an insulin-like growth factor receptor modulator.

70. The method of claim 69, wherein the amino acid sequence comprises a sequence selected from the group consisting of:

a. D815 (SEQ ID NO:2252); and

b. RP31-IGF (SEQ ID NO:2253).

71. A method of identifying an insulin-like growth factor receptor modulator comprising:

a. contacting insulin-like growth factor receptor with an amino acid sequence to form a complex,

b. contacting the complex of (a) with a compound library;

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c. identifying a compound which disrupts the complex of (a); and

d. determining whether the compound exhibits agonist or antagonist activity at insulin-like growth factor receptor, wherein this activity indicates identification of an insulin-like growth factor receptor modulator, and wherein the amino acid sequence comprises a sequence selected from the group consisting of:

- a. H2C-A-H6 (SEQ ID NO:2228);
- b. RP9 (SEQ ID NO:2229);
- c. C1 (SEQ ID NO:2230);
- d. L-RP9ex (SEQ ID NO:2231);
- e. RP33-IGF (SEQ ID NO:2232);
- f. RP6KK (SEQ ID NO:2233);
- g. RP30-IGF (SEQ ID NO:2234);
- h. RP43 (SEQ ID NO:2235);
- i. RP6 (SEQ ID NO:2236);
- j. RP9-RP9 (C-C; SEQ ID NO:2237);
- k. RP9-RP9 (C-N; SEQ ID NO:2238);
- l. RP9-L-RP9 (SEQ ID NO:2239);
- m. G33-RP9 (SEQ ID NO:2240);
- n. RP30-IGF-12-RP30-IGF (SEQ ID NO:2241);
- o. RP9-L-RP6 (SEQ ID NO:2242);
- p. G33-D8B12 (SEQ ID NO:2243);
- q. D8B12-12-RP9 (SEQ ID NO:2244);
- r. RP52 (SEQ ID NO:2245);

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- s. RP54 (SEQ ID NO:2246);
- t. RP33K-IGF (SEQ ID NO:2266);
- u. C1KK (SEQ ID NO:2266); and
- v. RP56 (SEQ ID NO:2247).

72. A method of identifying an insulin-like growth factor receptor modulator comprising:

- a. contacting insulin-like growth factor receptor with an amino acid sequence to form a complex,
- b. contacting the complex of (a) with a compound library;
- c. identifying a compound which disrupts the complex of (a); and
- d. determining whether the compound exhibits agonist or antagonist activity at insulin-like growth factor receptor, wherein this activity indicates identification of an insulin-like growth factor receptor modulator, and wherein the amino acid sequence comprises a sequence selected from the group consisting of:

- a. S175 (SEQ ID NO:2248);
- b. G33 (SEQ ID NO:2249);
- c. 12-RP9ex (SEQ ID NO:2250);
- d. L-RP9ex (SEQ ID NO:2231);
- e. D815 (SEQ ID NO:2252);
- f. RP31-IGF (SEQ ID NO:2253);
- g. RP9-12-RP9 (SEQ ID NO:2254);
- h. D112-12-D112 (SEQ ID NO:2255);
- i. G33-6-G33 (SEQ ID NO:2256);
- j. RP9-L-RP6 (SEQ ID NO:2242);

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- k. D112-12-RP30-IGF (SEQ ID NO:2257);
- l. RP9-L-RP6 (SEQ ID NO:2242);
- m. RP30-IGF-12-D112 (SEQ ID NO:2258);
- n. RP6-RP9 (SEQ ID NO:2259);
- o. D8B12-12-RP9 (SEQ ID NO:2244);
- p. D815-RP9 (SEQ ID NO:2260);
- r. RP48 (SEQ ID NO:2261);
- s. RP6-L-D8B12 (SEQ ID NO:2263);
- t. RP30-IGF-12-RP31-IGF (SEQ ID NO:2264);
- u. RP31-IGF-12-RP30-IGF (SEQ ID NO:2265); and
- v. RP60 (SEQ ID NO:2262).

73. A method of identifying an insulin-like growth factor receptor modulator comprising:

a. screening a library of amino acid sequences to isolate an amino acid sequence that binds to an insulin-like growth factor receptor, wherein the library is derived from a peptide sequence comprising at least one formula sequence selected from the group consisting of Formula 1, Formula 2, and Formula 6; and

b. determining whether the amino acid sequence isolated in (a) exhibits agonist or antagonist activity at insulin-like growth factor receptor in an insulin-like growth factor-responsive cell selected from the group consisting of FDC-P2, MCF-7, and MiaPaCa, wherein this activity indicates identification of an insulin-like growth factor receptor modulator.

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74. A method of enhancing survival of an insulin-like growth factor-responsive mammalian cell comprising: contacting the cell with an amino acid sequence in an amount sufficient to enhance the survival of the cell, wherein the amino acid sequence is an insulin-like growth factor receptor agonist comprising at least one formula sequence selected from the group consisting of Formula 1, Formula 2, and Formula 6.

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGF ₃ R	IR	IGFR/IR	IR/IGFR
R40-3-40B2-IR	IRDMHYVWVQDRDRYINGVRQWYISDRYNPGSAFYRWFD	--	--	--	--	--
R40-4-40B12-IR	RMGLQALAHYRKSAGPIFLSSGSGVIKSGEGDFFYAWFRLQ	40.3	9.0	2.0	4.5	0.2
R40-4-40G11-IR	MPVSLFRRVWDYRDGEHETLESHYVVPQAALDKLFYSWFS	60.4	12.9	2.0	6.5	0.2
		52.6	37.5	2.0	18.8	0.1

FIG. 1A

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGF ₃ R	IR	IGFR/IR	IR/IGFR
R40-3-D5-IGFR	PLYGGGIHLIYYPGTMGYPGPPRQVKVLGDADKNFYDWF	--	--	--	--	--
R40-3-A6-IGFR	YRGMVLVLRISDGAGKVASEPPIRGQKVFAVNFYDWFV	--	--	--	--	--
R40-X-R35-IGFR	SGCCRLGLRWMFIVIGWSGALVCQSAASAAGFYDWFV	--	--	--	--	--

FIG. 1B

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag --	IGF β R --	IR --	IGF β R/IR --
R20 α -3-20D3-IR	IGGQGHQDGNFYDNFVEALA	46.3	36.2	7.0	5.2
R20 α -3-20F1-IR	VFWNCRSQQLDFYEFWEQAA	49.0	26.0	2.8	9.3
R20 α -3-20H1-IR	RVAGAISAPGLVSNKQDGLFYSWFRE	45.6	35.3	3.3	10.7
R20 α -3-20D1-IR	VLQARHGCDSDVSDCFYEWFA	50.8	37.5	3.0	12.5
R20 β -4-B12-IR	GAFYRWFHEALVGSERVPDV	41.9	2.9	5.7	0.5
R20 β -4-H3-IR	HEAFYDWFSAALVDGGYELMG	13.9	5.8	2.4	2.4
R20 β -4-D10-2-IR	RIGGGWARSEGFYEFVREL	21.5	7.3	2.9	2.5
R20 β -4-C8-IR	LPAGGA?GFA?RGFYEFES	44.9	31.1	9.6	3.2
R20 β -4-E7-IR	GHSWALVRHVDRLFYEFWFDL	45.0	18.8	5.9	3.2
R20 β -4-E7-2-IR	LGTSAGQGVGHRAFYQWFQS	45.0	18.8	5.9	3.2
R20 β -4-G3-IR	RGGTFFYEFESALRKHGAG	38.6	7.5	2.0	3.8
R20 β -4-H6-IR	NSSGQVVGTLTFYSWFASQV	14.8	7.6	2.0	3.8
R20 β -4-G11-IR	FYGFWSRQLSLTPRDDWGLP	39.4	7.5	1.9	3.9
R20 β -4-G8-IR	RMFYEFWSQMGAGPTEGSA	41.2	15.1	3.4	4.4
R20 β -4-H9-IR	IGGQGHQDGNFYDNFVEALA	43.1	8.8	2.0	4.4
R20 β -4-H8-IR	RDKPTDQEEQNWSFYEFWRH	47.9	43.7	9.3	4.7
R20 β -4-B8-IR	WSALLSVMDTGFYAWFDDAV	44.0	40.1	8.4	4.8
R20 β -4-E2-IR	SRDQTNFTNSAGFYGWFER	16.3	13.9	2.4	5.8
R20 β -4-F4-IR	GVGTLTMSSDAFYTWV	15.3	5.9	1.0	5.9
R20 β -4-A8-IR	IGGSFVEFYGFNFNDQV	43.3	36.0	6.0	6.0
R20 β -4-C4-IR	DIGSDGHGRRWDSFYRWFEM	17.3	26.8	4.3	6.2
R20 β -4-D7-IR	VLQARHGCDSDVSDCFYEWFA	44.8	36.2	5.6	6.5
R20 β -4-D2-IR	DPERMQSDVGFYEFWFAAVG	31.2	29.4	2.9	10.1

FIG. 1C

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF ₃ R	IGFR/IR	IR/IGFR
R20-4-B9-IGFR	DPERMQSDVGFYEWFRRAAVG	40.1	16.6	--	--
R20-4-F8-IGFR	DIGSDGHGRRWDSFYRWFEM	39.2	13.9	--	--
R20-4-G12-IGFR	PFYQWFLDQSVGSGRGGGLR	36.7	8.0	--	--
R20-4-D10-IGFR	AVAPLSVRGRDSGFYSWFSS	40.2	4.1	--	--

FIG. 1D

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR
A6S-3-E12-IR	XXXXXXXXXXXXNFYDFVXXXX	26.2	1.3	8.0	0.2
A6S-2-C1-IR	GRVDWLQRNANFYDFWFAELG	41.2	1.3	7.0	0.2
A6S-1-A7-IR	RMYSTGAPQNFYDFWVQEW	47.2	2.3	11.1	0.2
A6S-2-C8-IR	HHTQGLQVQRNFYDFWVNELR	44.9	1.5	5.5	0.3
A6S-3-E10-IR	MHRMQHDGTSNFYDFWFLQWA	46.9	1.6	5.0	0.3
A6S-2-D5-IR	AMHVVAQGGPNFYDFWVRELR	31.9	1.2	3.7	0.3
A6S-1-B2-IR	AIQMNGNLAFNFYDFWVRELT	31.6	1.8	5.3	0.3
A6S-1-A4-IR	TDRKSVQEPNRFYDFWVNAAR	43.3	3.6	9.2	0.4
A6S-4-G3-IR	PHGHRGFAQSNFYDFWVTQEE	31.3	2.3	5.1	0.5
A6S-4-H8-IR	RLASASVPGQNFYDFWVDQLL	11.5	1.7	3.6	0.5
A6S-3-E11-IR	RQSEFSTLNSNFYDFWVRELE	26.3	2.3	4.4	0.5
A6S-1-A1-IR	GQAQLSIRDVNFYDFWVQQLV	36.9	3.7	6.5	0.6
A6S-2-C9-IR	MSEPAGVGNFNFYDFWVFAQLF	43.6	1.3	2.3	0.6
A6S-2-C4-IR	VGTRARLDRNFYDFWVFGQYS	34.5	5.6	9.6	0.6
A6S-4-H10-IR	SREAVQKRANFYDFWVQQLS	39.2	4.4	6.9	0.6
A6S-4-G7-IR	LAQFAGSRNQNFYDFWVEQLG	19.1	1.4	2.2	0.6
A6S-4-H2-IR	GQEYFDQMGLNFYDFWVRELD	25.5	2.6	3.9	0.7
A6S-2-C3-IR	RQSPQPPHGSNFYDFWVEAIN	31.1	1.6	2.4	0.7
A6S-2-C11-IR	LMQSLGSGSTNFYDFWVQQMV	20.9	3.3	4.6	0.7
A6S-3-F3-IR	DQORSACDGTNFYDFWVCQLS	37.1	3.0	4.2	0.7
A6S-3-E5-IR	LDGTKACQVRNFYDFWVCQTE	31.6	2.5	3.5	0.7
A6S-1-B7-IR	PEARRTVVHSNFYDFWVFAQLS	49.2	1.6	2.3	0.7
A6S-3-E7-IR	PWMLSVGIQDNFYDFWVGLDS	37.2	5.0	6.3	0.8
A6S-4-G6-IR	ASHQRGSSDNFYDFWVFAQMR	16.8	3.1	4.0	0.8
A6S-2-C2-IR	TLEREGEFSGNFYDFWVEQLH	29.7	2.4	3.1	0.8
A6S-3-F1-IR	DRQSIGSVHGDFYDFWVFSALG	29.7	2.3	3.0	0.8
A6S-2-C5-IR	DWDKLGSLSENFYDFWVDQLA	42.9	6.1	7.0	0.9
A6S-3-E4-IR	VRVVLNQSGRNFYDFWVFIQLE	20.9	2.1	2.3	0.9
	MASWQSRTPDNFYDFWVRELS				

FIG. 1E-1

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF3R	IGFR/IR	IR/IGFR
A6S-3-E9-IR	XXXXXXXXXXNFYDFVXXXX	36.6	9.0	8.9	1.0
A6S-3-E1-IR	TTCHPRGEDCNFYDFVQLR	36.7	6.8	6.9	1.0
A6S-4-H12-IR	VRGNDVLRANFYDFVDQLS	46.3	6.1	5.8	1.0
A6S-2-D3-IR	TPRSQVRSDHNFYDFVYQLA	37.0	5.3	5.1	1.0
A6S-3-E8-IR	ESLTGSRPDRNFYDFVQOTS	42.7	5.2	5.1	1.0
A6S-1-A12-IR	PQSLTEVRTGNFYDFVVLH	39.7	2.1	2.1	1.0
A6S-4-H3-IR	DVGMGRVKETNFYDFVRLI	18.6	3.1	2.9	0.9
A6S-3-F7-IR	GADDIRSLNTNFYDFVNQLS	46.2	2.3	2.1	0.9
A6S-2-D8-IR	GVSIOAGYKTNFYDFVEAVR	31.2	2.0	1.7	0.9
A6S-3-F10-IR	VGEHRQMSVGNFYDFVMQIA	39.0	5.9	4.5	0.8
A6S-4-G11-IR	GSSLGRSGPGNFYDFVDQLE	44.8	4.3	3.3	0.8
A6S-2-D2-IR	HRQQDVVRQGNFYDFVQALE	33.5	3.6	2.7	0.8
A6S-4-G8-IR	QDTFLTAREGNFYDFFIRALE	11.1	2.5	1.9	0.8
A6S-4-H6-IR	EAIMREEGQANFYDFVFRQLE	22.4	2.4	1.9	0.8
A6S-2-D10-IR	VCDVSTGGTNFYDFVFCQVG	41.3	2.1	1.7	0.8
A6S-3-F4-IR	PQPRSASTPLNFYDFVQATG	37.0	13.5	9.9	0.7
A6S-4-G9-IR	GVSRGSGGDPNFYDFVFMQLR	36.2	11.8	7.8	0.7
A6S-3-F5-IR	GPRHDSSRGNFYDFVEQLA	48.1	7.2	4.8	0.7
A6S-4-H1-IR	ERFALEVQGSNFYDFVFRQVI	18.3	3.6	2.6	0.7
A6S-3-F6-IR	NLKSSATVGGNFYDFVEQQL	18.7	2.9	1.9	0.7
A6S-3-F11-IR	MEGPPAGGPLNFYDFVFAQVD	33.8	2.0	1.4	0.7
A6S-2-C6-IR	RLDVAGHRGGNFYDFVFKQLH	46.7	19.2	12.1	0.6
A6S-4-G4-IR	PWSDHEALNQNFYDFVFSQVL	36.9	18.2	10.7	0.6
A6S-4-G12-IR	EDRLNGESTNFYDFVFRQLA	32.8	12.8	7.9	0.6
A6S-2-D7-IR	GKLVASTLDDNFYDFVFRQLS	33.2	12.0	7.1	0.6
A6S-4-G10-IR	SGPVVQTQNGNFYDFVHQLR	33.9	10.8	6.8	0.6
A6S-3-F9-IR	VDRAGPAGSDNFYDFVFAOLD	44.3	9.6	5.7	0.6
A6S-3-F2-IR	SLGRNDRPDENFYDFVFSQVQ	23.2	4.3	2.5	0.6
	RVMATANAPMNFYDFVVLQ				

FIG. 1E-2

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGF ₃ R	IR	IGFR/IR	IR/IGFR	
A6S-4-G1-IR	XXXXXXXXXXXXXXXXNFYDWFVXXXX	--	--	--	--	--	--
A6S-1-A3-IR	NGVERAGTGDNFYDWFVQAQLH	36.2	31.8	15.7	2.0	0.5	0.5
A6S-3-F12-IR	PFAGKGDKTGNFYDWFVSLTG	39.9	12.6	6.0	2.1	0.5	0.5
A6S-4-G2-IR	GMPQEYMDQVNFYDWFVQAQVD	41.4	7.4	4.0	1.9	0.5	0.5
A6S-1-B1-IR	MGTPAVGDBGANFYDWFVVRQLG	26.7	7.0	3.5	2.0	0.5	0.5
A6S-2-D11-IR	SKCKAWYGANNFYDWFVWQVD	30.6	3.7	1.9	1.9	0.5	0.5
A6S-2-D1-IR	EAASLGSDRNFYDWFVRQVV	48.4	37.4	13.5	2.8	0.4	0.4
A6S-3-E2-IR	VERSASSQDGNFYDWFVVQIR	37.8	30.6	12.0	2.6	0.4	0.4
	TSEVQRRSQDNFYDWFVQAQVA	33.1	24.7	9.8	2.5	0.4	0.4

FIG. 1E-3

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
A6S-4-E4-IGFR	XXXXXXXXXXNFYDFVXXXX	--	--	--	--
A6S-2-D2-IGFR	ERSAAGFREGFYDFVQAQVN	27	32	--	--
A6S-2-F2-IGFR	RAERGSMDNSFYDFVQQLP	36	30	--	--
A6S-4-F3-IGFR	LAMSVASRPANFYDFVQAIV	35	30	--	--
A6S-4-G4-IGFR	HNSSPMRTGNFYDFVQELR	26	30	--	--
A6S-4-G3-IGFR	SALSGPVQPINFYDFVFTGM	26	30	--	--
A6S-2-H2-IGFR	GAQAIREIHHNFYDFVQAQVT	21	29	--	--
A6S-2-E3-IGFR	RGQRESDSGTNFYDFVGAIR	40	28	--	--
A6S-4-C6-IGFR	VQEGLSGMEGNFYDFVDQLF	36	28	--	--
A6S-4-F5-IGFR	RLDRSSTSGNFYDFVQAQVG	25	28	--	--
A6S-4-H3-IGFR	GSQHSGREPHNFYDFVQAQVG	24	28	--	--
A6S-4-H4-IGFR	GRGDQRHETTNFYDFVRELO	20	28	--	--
A6S-2-H1-IGFR	PRMVEKPSDNFYDFVTQLS	20	28	--	--
A6S-4-E6-IGFR	RVGIQVDPHTNFYDFVFIQLT	42	27	--	--
A6S-4-B6-IGFR	RSSGGLLSQGNFYDFVFSQLE	24	26	--	--
A6S-4-D2-IGFR	SDARQAGLQENFYDFVFSQVR	23	26	--	--
A6S-4-G5-IGFR	PPYRSSRLGENFYDFVFMQVR	19	26	--	--
A6S-2-A3-IGFR	QEVTRTRDDKNFYDFVFSQIF	18	26	--	--
A6S-4-E2-IGFR	SRAPYGSTAGNFYDFVQAVS	37	25	--	--
A6S-4-G6-IGFR	?DGQSVSSKGNFYDFVQQMT	25	25	--	--
A6S-4-G2-IGFR	RLMGGIAEPQNFYDFVREVA	20	25	--	--
A6S-4-D6-IGFR	SAGHMPRESNFYDFVDQVV	25	24	--	--
A6S-4-F4-IGFR	LGAETWDGINFYDFVFKQVS	22	24	--	--
A6S-4-C3-IGFR	VGHSGVPPYPNFYDFVFMQVS	22	24	--	--
A6S-4-H5-IGFR	VTMLDKGAQDNFYDFVREVA	21	24	--	--
A6S-4-H6-IGFR	HHSPGNEHGYNFYDFVQLQA	19	24	--	--
A6S-4-F6-IGFR	GSIAQLIMRANFYDFVFEQTN	18	24	--	--
A6S-3-H1-IGFR	LKGSSQPLSVNFYDFVQQIK	17	24	--	--
	PASNKNSLAENFYDFVQQTR	30	23	--	--

FIG. 1F-1

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Ratios over Background		Comparisons				
Clone	Design	Sequence	E-Tag	IGF ₃ R	IR	IGFR/IR IR/IGFR
A6S-4-A6-IGFR		XXXXXXXXXXNFYDFVXXXX	--	--	--	--
A6S-4-E3-IGFR		HVEHMAVGDNFYDFVQQLR	21	23	--	--
A6S-4-D3-IGFR		RGMTGMVGRGNFYDFVQQLR	21	23	--	--
A6S-3-E10-IGFR		GLRSEQGNRLNFYDFVQAIA	20	23	--	--
A6S-4-D1-IGFR		RVREKLPRPNFYDFVFNQIH	23	22	--	--
A6S-4-B2-IGFR		SNPSRQDASVNFYDFVREVA	22	22	--	--
A6S-4-A2-IGFR		QSVDLSPDSNFYDFVEVLS	21	22	--	--
A6S-4-A5-IGFR		IGGQGHQDGNFYDFVEALA	20	22	--	--
A6S-4-C1-IGFR		VEVQRHIRKDNFYDFVVKID	19	22	--	--
A6S-4-B1-IGFR		CWARPCGDAANFYDFVQQAS	16	22	--	--
A6S-4-B4-IGFR		RHERGKEGPNFYDFVSQVV	19	21	--	--
A6S-4-D4-IGFR		ERSPRPALASNFYDFVQQVV	19	21	--	--
A6S-3-F8-IGFR		IARMRETFQPNFYDFVDQLA	18	21	--	--
A6S-3-H9-IGFR		GRGQGLKRPDNFYDFVAAAK	25	20	--	--
A6S-3-G2-IGFR		YSIEVQDWNENFYDFVSQLG	23	20	--	--
A6S-4-H2-IGFR		TWNWEERKQDNFYDFVQGOLK	21	20	--	--
A6S-4-A3-IGFR		VTFTSAVFHENFYDFVVRQVS	19	20	--	--
A6S-3-G10-IGFR		LAINDLVTHKNFYDFVDQLR	18	20	--	--
A6S-3-E5-IGFR		GAVGLAEAGPNFYDFVSQVQ	24	19	--	--
A6S-3-H2-IGFR		RYRGERHDGRNFYDFVEQVN	21	19	--	--
A6S-3-G3-IGFR		QGAEGRLSEGNFYDFVQAVS	21	19	--	--
A6S-4-H1-IGFR		PRLHMGSMDGFYDFVQIA	21	18	--	--
A6S-4-G1-IGFR		IVAGARHSEVNFYDFVVIQVR	18	18	--	--
A6S-4-A1-IGFR		AELVGAGVRGNFYDFVDQLV	16	16	--	--
A6S-2-F1-IGFR		DSSRLWLGERNFYDFVQAIS	17	12	--	--
A6S-2-G1-IGFR		VGQVGRYVRSNFYDFVQQAM	30	8	--	--
A6S-1-C5-IGFR		RPQLVESGSKNFYDFVQVVR	30	8	--	--
A6S-2-B2-IGFR		RIHNQTERGGNFYDFVHQLV	27	7	--	--
		EMYGDTSERVNFYDFVSAIQ	30	5	--	--

FIG. 1F-2

Ratios over Background		Sequence	Comparisons				
Clone	Design		E-Tag	IGF ₃ R	IR	IGFR/IR	IR/IGFR
A6S-1-D5-IGFR		XXXXXXXXXXXXNFYDWFVXXXXX	--	--	--	--	--
A6S-1-A2-IGFR		RVGSGMEDLGNFYDWFVRQAQ	25	5	--	--	--
A6S-3-E6-IGFR		KDPVTVSQGRNFYDWFVVIQ	20	5	--	--	--
A6S-1-G3-IGFR		DARDHGVWMSNFYDWFVAQVS	20	5	--	--	--
A6S-3-G4-IGFR		VATVHVGGGMNFYDWFVAQVG	19	5	--	--	--
A6S-3-H8-IGFR		CADPGACSSLNFYDWFVQMRG	21	4	--	--	--
A6S-3-E3-IGFR		NPTSVQQYGVNFYDWFVNLS	20	4	--	--	--
A6S-3-D9-IGFR		RPSLPEVRPGNFYDWFVQSVR	19	4	--	--	--
A6S-2-A1-IGFR		SLQGADFQQGNFYDWFVSELA	17	4	--	--	--
A6S-1-H4-IGFR		LSSRGRVTMRNFYDWFVAQVV	31	3	--	--	--
A6S-3-C1-IGFR		HKSWTTMSPLNFYDWFVAQVE	18	3	--	--	--
A6S-3-B10-IGFR		RPVIGGGGTRNFYDWFVAQMI	17	3	--	--	--
		YDQDPPYWGLNFYDWFVREVA	16	3	--	--	--

FIG. 1F-3

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Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
A6L-3-D1-IR		<u>YRGMLVLGRISDGAGKVAS</u> EP ^{PAR} IG ^{QK} VFAVNFYDW ^{FV}	19.0	.4.0	--	--	--
A6L-4-H7-IR		<u>Q</u> RGMLVRGRISHGAGKIAEPPDCLGQKACAVNFYDW ^{FV}	22.6	19.8	26.5	0.7	1.3
A6L-4-H4-IR		<u>Q</u> RGMLLLGRISDDAGKVA ^{SE} PSARRGQKVFAFNFYDW ^{FV}	37.5	3.5	4.2	0.8	1.2
A6L-4-E4-IR		YRGILVLGRIS ^{EG} AGKVA ^{SE} PAARIG ^{QK} VFAFNFYDW ^{FV}	38.5	21.1	25.8	0.8	1.2
A6L-4-G7-IR		<u>Q</u> RGMLALGRISDGAGKVA ^{SE} PPAGIG ^{QK} VFAFNFYDW ^{FV}	38.1	5.4	6.0	0.9	1.1
A6L-3-C3-IR		FRRLVLGHFSDGAGKVG ^{SE} PAARIG ^{QK} VFDVNFYDW ^{FV}	38.6	16.2	18.5	0.9	1.1
A6L-3-B6-IR		YRGMLVLGRISDGAGKVA ^{SE} PPARIG ^Q EVFADNFYDW ^{FV}	34.7	21.8	23.1	0.9	1.1
A6L-4-G11-IR		YRGMLVLGRISDGAGEVA ^{SE} PPARIG ^Q EVFALNFYDW ^{FV}	33.1	27.8	30.3	0.9	1.1
A6L-4-G12-IR		VPWYAGSGSSSDGAGKVA ^{SE} PPARID ^{QK} VFAVNFYDW ^{FV}	27.6	2.0	2.0	1.0	1.0
A6L-3-A10-IR		YRGQLVLGRISYGAGKVGCDPPARIG ^{QK} DWAVNFYDW ^{FV}	32.0	2.3	2.3	1.0	1.0
A6L-4-E12-IR		<u>Q</u> RGMLVLGRFSDGAGNVA ^{SE} PPAGIG ^Q EVFPVNFYDW ^{FV}	21.1	2.4	2.4	1.0	1.0
A6L-4-E10-IR		<u>Q</u> RGMLVLGRISDGAGKVA ^{AE} PPDCLGQKVCANFYDW ^{FV}	3.1	2.4	2.4	1.0	1.0
A6L-4-G8-IR		<u>Q</u> RGMRVLGRISDGAGKVA ^{SE} LP ^{PR} IG ^{QK} DFAVNFYDW ^{FV}	30.1	3.8	3.8	1.0	1.0
A6L-3-C12-IR		<u>Q</u> RGMLVLGRISDGAGKVA ^{YE} APARIG ^Q TVFAVNFYDW ^{FV}	37.9	4.7	4.7	1.0	1.0
A6L-4-H11-IR		<u>Q</u> PWCAGSGRIYDGACKVA ^{SE} PPAHIG ^Q EVFAVNFYDW ^{FV}	29.5	5.7	5.7	1.0	1.0
A6L-4-F10-IR		<u>Q</u> RGMLVLDGRISDGAGKVASGPPARIG ^Q NVLAVNFYDW ^{FV}	35.4	9.6	9.6	1.0	1.0
A6L-4-E9-IR		YRGMLVVGRISDGTGKVASQPPARIG ^{QK} VFAVNFYDW ^{FV}	31.6	10.5	10.5	1.0	1.0
A6L-4-H8-IR		YRGMLVLGRISDGAGKVASVPPAHIG ^{QK} VFAFNFYDW ^{FV}	39.8	12.9	12.9	1.0	1.0
A6L-3-A11-IR		<u>Q</u> HGMLVLGRVSVGAGKVPSEPPQARIGHKVF ^{DN} FYDW ^{FV}	38.2	14.6	14.6	1.0	1.0
A6L-4-F9-IR		YSGYAGSGSFSDGAGKVA ^{SE} PPARIS ^Q EVLADNFYDW ^{FV}	29.0	17.5	17.5	1.0	1.0
A6L-4-G2-IR		YRGMLVLGRISDGAGKVA ^{SE} PPARIG ^{QK} VSAVNFYDW ^{FV}	35.7	18.4	18.4	1.0	1.0
A6L-4-E8-IR		YHGKLDLGRISVGVGKVA ^{SE} PPARIG ^{QK} VFADNFYDW ^{FV}	29.5	21.4	20.7	1.0	1.0
A6L-4-H10-IR		YRGQAGSGVGLTVAGKVASDPPARIG ^{QK} VFADNFYDW ^{FV}	28.7	21.6	21.6	1.0	1.0
A6L-4-G9-IR		HRGMLVLGRISEGAGNVDP ^{EP} PARIG ^Q NVFAFNFYDW ^{FV}	30.0	22.1	22.1	1.0	1.0
A6L-4-F7-IR		<u>Q</u> RGMPVLGRISDGAGKVG ^{SE} PPARIA ^{RK} VFPVNFYDW ^{FI}	37.1	22.6	22.6	1.0	1.0
A6L-4-E11-IR		<u>Q</u> GGLLVTGRISDGAGKVA ^{SE} PPGGIG ^{QK} VFAFNFYDW ^{FV}	28.6	23.6	24.4	1.0	1.0
		YPWYGGSGTYLDGAGKVA ^{SE} PPARID ^Q QVFAFNFYDW ^{FV}	38.4	26.5	26.5	1.0	1.0

FIG. 1G-1

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Clone	Parental/Design	Sequence	Ratios over Background			Comparisons		
			E-Tag	IGF β R	IR	IGF β R/IR	IR/IGF β R	IR/IGF β R
		<u>YRGMVLGRISDGAGKVASEPPARIGQKVFAVNFYDWFV</u>	19.0	4.0	--	--	--	--
A6L-4-H9-IR		YRAMLVLRISDVAGIVDSEPTTRIGQKVFAVNFYDWFV	37.5	27.3	27.3	1.0	1.0	1.0
A6L-4-E1-IR		YRGMVLGRISQAGNVASEPSSRIGQKVFAVNFYDWFV	35.4	32.6	31.4	1.0	1.0	1.0
A6L-3-A5-IR		YRGMVLGRISDGAGKVDEYPPARIGQKVFAVNFYDWFV	38.3	34.6	35.5	1.0	1.0	1.0
A6L-4-G4-IR		YRGMGLGGISAGAGIVASEPPARVGVQKVFAVNFYDWFV	30.4	17.7	15.2	1.2	0.9	0.9
A6L-4-H2-IR		YRGILFQGRIPDGAGKVASEPPTTRIGERVFAVNFYDWFV	36.1	4.2	3.6	1.1	0.9	0.9
A6L-4-E6-IR		QGGMPVLGRISDGAGKVAFEPPARIGQKVFAVNFYDWFV	28.6	24.1	22.7	1.1	0.9	0.9
A6L-4-H5-IR		YRGMVLGRIQDGAGKVASEPPARIGQKVFAVNFYDWFV	37.2	24.6	23.1	1.1	0.9	0.9
A6L-4-H3-IR		QRGMLVLGGVSDGAGKVASDPPASIGQNVFAVNFYDWFV	37.1	9.1	7.2	1.3	0.8	0.8
A6L-4-E5-IR		YPGMLILDRISDGASKVSEPPASIGQKVFAVNFYDWFV	42.1	30.6	24.4	1.3	0.8	0.8
A6L-3-C5-IR		YRGMVLDRISDGAGKVASEQPARIGQEVYAVNFYDWFV	42.2	21.9	17.5	1.2	0.8	0.8
A6L-4-G6-IR		YRGMIDLGRISGGVGKVAESPARIGQKVFAVNFYDWFV	29.8	4.3	2.8	1.5	0.7	0.7
A6L-3-D4-IR		QRGMLVLGRISDGAGEVASEKVFAVNFYDWFV	39.9	12.4	8.4	1.5	0.7	0.7
A6L-3-A7-IR		QRGMLVLGRVSDGAGKVDSAPPARIGQKVFAVNFYDWFV	31.0	21.2	14.0	1.5	0.7	0.7
A6L-3-A6-IR		QRGMLVLGRMSDGAGKVAFEPPARIGQGVFAVNFYDWFV	25.5	12.3	8.8	1.4	0.7	0.7
A6L-4-E7-IR		QRGTLVLGRISDGAGKAASEPPARIGQNVFAVNFYDWFV	38.4	12.5	7.1	1.7	0.6	0.6
A6L-3-C6-IR		QRGMLVLDRISDGAGKVAEPPARIGQKVFAVNFYDWFV	28.8	10.9	6.7	1.6	0.6	0.6
A6L-4-F5-IR		QRGMLVLGRISDGAGEVASEPPARIGEVYAVNFYDWFV	33.8	6.3	4.1	1.5	0.6	0.6
A6L-3-B7-IR		QRGILVRGRISDGAGKVGESEPPARISGEKVFAVNFYDWFV	27.6	9.4	5.0	1.9	0.5	0.5
A6L-4-F4-IR		QLGMVVLGRISDGSKAASEPPARISQKVFAVNFYDWFV	38.9	17.6	9.4	1.9	0.5	0.5
A6L-4-E3-IR		QRGMLVLGRISDGDGKVASEPPARIGQKVFAVNFYDWFV	38.0	6.9	3.8	1.8	0.5	0.5
A6L-0-E6-IR		YRGMVLGRSSDGAGKVAFERPARIGQTVFAVNFYDWFV	31.0	31.0	1.8	17.0	0.1	0.1
A6L-0-E4-IR		YRGMVLGRISDGAG#VASEPPARIGRKVFAVNFYDWFV	26.0	16.0	1.3	13.0	0.1	0.1
A6L-0-H3-IR		YRGMVLGRISGGAGKAA SERPARIGQKVSAVNFYDWFV	27.0	26.0	2.0	13.0	0.1	0.1

FIG. 1G-2

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Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
A6L-4-F8-IGFR	YRGMLVLGRISDAGAGKVA	SEPPARIGQKVFVNFYDWFV	19	4	--	--	--
A6L-2-G9-IGFR	YRGMLVQGRISDAGAGKVAS	VPVRIGQKVI	26	28	--	--	--
A6L-4-E7-IGFR	YRGRLGLGRISDVAGKACD	PSARIGQKVL	39	22	--	--	--
A6L-4-G10-IGFR	YRGMLVLGRISDAGAGKVA	SEPPARIGQKVFVNFYDWFV	23	22	--	--	--
A6L-2-E9-IGFR	QGGMLVPGRISDAGAGKVA	SEPPARIGPKGFAGNFYDWFV	19	22	--	--	--
A6L-2-D6-IGFR	YRGMRVLGRISDAGAGKVA	SEPPTHIGQKVPVNFYDWFV	38	21	--	--	--
A6L-3-H12-IGFR	YRGMLVLGRISDAGAGKVA	SEPPARIGQKVFVNFYDWFV	34	21	--	--	--
A6L-4-A7-IGFR	YRGQGMVLGRISDAGAGKVA	SEPPGRIGQKVPVNFYDWFV	24	21	--	--	--
A6L-4-B8-IGFR	YRGMLGLGRITGGAGKVA	SEPPDRIGQHVFDNFYDWFV	20	20	--	--	--
A6L-4-G7-IGFR	DGMLVLGRISDAGAGKVA	SEPPARIGQKVFVNFYDWFV	20	19	--	--	--
A6L-2-D9-IGFR	YRGMRVRGRISDAGAGKAA	SDPRARIGQTVLDNFYDWFV	19	19	--	--	--
A6L-4-F7-IGFR	YRGMWVLGRISYAGAGKVA	YEPPARMGQKGFVNFYDWFV	38	18	--	--	--
A6L-4-E12-IGFR	YRGMLVGGRIAGGAGI	VASEPPARIGQKVFVNFYDWFV	18	18	--	--	--
A6L-4-H7-IGFR	YRGLLGLGGRISDAGAGKVA	SEPPARNQKVFVNFYDWFV	15	13	--	--	--
A6L-4-H12-IGFR	YRGMLGLGRISAGAGKVA	SGAPARIGQEDFVNFYDWFV	14	13	--	--	--
A6L-2-A4-IGFR	YRGMLALGRISDAGAGKVA	SEPPARIGQNVFVNFYDWFV	13	12	--	--	--
A6L-3-D10-IGFR	YRGMLVLGRISDAGAGKVA	SEPPARIGQKVLVNFYDWFV	17	4	--	--	--
A6L-2-F6-IGFR	YPGMLVPGRISDAGAGKVA	SEPPARIGQKVFVNFYDWFV	16	4	--	--	--
A6L-2-B11-IGFR	YRGMLVPGRISDAGAGKVA	SEPPARIGQKVFVNFYDWFV	15	4	--	--	--
A6L-1-B7-IGFR	YRGVLVGRVSDGVGKVA	SEPPAHRGQKVFVNFYDWFV	26	3	--	--	--
A6L-1-D8-IGFR	YRRMLVLGRISDGAANVA	SGPPDRIGQKVFAGNFYDWFV	23	3	--	--	--
A6L-0-A11-IGFR	YRRMLALGRFSDVTGDVA	SEPPAHIGQKVVAVNFYDWFV	23	3	--	--	--
A6L-3-B7-IGFR	YRGMVVRGRIFDGPVKVA	SEPPARIGQKVFVNFYDWFV	19	3	--	--	--
A6L-1-G7-IGFR	YRGMLILGRISDAGAGKVA	SEPPARVQDVVAVNFYDWFV	9	3	--	--	--
A6L-1-E9-IGFR	YRGRLVGGRISDGVGKVA	SEPPGRIGQKVFVNFYDWFV	20	2	--	--	--
A6L-1-C9-IGFR	QRGLLVGRIFDAGAGKVA	SDPPARIGQKDFADNFYDWFV	18	2	--	--	--
A6L-0-G10-IGFR	YRGMLVLGRISDAGAGKVA	FEPPARIGQNVFVNFYDWFV	18	2	--	--	--
A6L-1-G8-IGFR	YRCMPVLGRISDAGAG#VA	SDRPARIGQKVFVNFYDWFV	18	2	--	--	--
	YRGRLVLGRISDAGAGKVA	EAEPASMDSKVFAGNFYDWFV	15	2	--	--	--

FIG. 1H

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Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag --	IGF β R --	IR --	IGF β R/IR --	IR/IGF β R --
E4D α -1-B8-IR	GFREGQRYWFWVAQVT	40.7	1.0	12.3	0.1	12.3
E4D α -3-E5-IR	GFREGYFYDWFLAQVT	39.6	2.0	1.5	1.3	0.8
E4D α -1-A1-IR	GFREGDFYEWFWVAQVT	48.7	44.9	31.4	1.4	0.7
E4D α -2-D9-IR	GFREGQFYEWFAAQVT	22.9	3.3	2.4	1.4	0.7
E4D α -1-B3-IR	GFREGTFYDWFWVAQVT	41.8	38.6	26.5	1.5	0.7
E4D α -1-A6-IR	GFREGNFYDWFEAQVT	56.3	51.2	32.6	1.6	0.6
E4D α -1-A10-IR	GFREGAFYDWFEAQVT	48.9	42.2	26.5	1.6	0.6
E4D α -1-A8-IR	GFREGAFYDWFWVAQVT	46.9	41.5	26.2	1.6	0.6
E4D α -1-B1-IR	GFREGKFYQWFEAQVT	44.1	31.1	19.7	1.6	0.6
E4D α -2-C9-IR	GFREGDFYDWFWAQVT	34.0	8.1	4.8	1.7	0.6
E4D α -1-A3-IR	GFREGTFYEWFWVAQVT	45.3	40.3	22.5	1.8	0.6
E4D α -1-A9-IR	GFREGNFYDWFWVAQVT	46.9	41.0	22.5	1.8	0.5
E4D α -3-F3-IR	GFREGQFYEWFLAQVT	37.2	14.1	8.0	1.8	0.6
E4D α -2-D3-IR	GFREGQFYDWFLAQVT	35.1	16.3	8.7	1.9	0.5
E4D α -2-D6-IR	GFREGDFYDWFWAQVT	33.2	5.6	2.8	2.0	0.5
E4D α -3-F10-IR	GFREGQFYDWFWAQVT	27.8	4.5	2.3	2.0	0.5
E4D α -2-D5-IR	GFREGYFYEWFWAQVT	43.8	23.8	11.4	2.1	0.5
E4D α -3-F4-IR	GFREGDFYQWFEAQVT	25.9	7.6	3.7	2.1	0.5
E4D α -3-E3-IR	GFREGSFYGFWFWAQVT	34.6	4.0	1.9	2.1	0.5
E4D α -3-F8-IR	GFREGSFYAWFWAQVT	20.9	16.0	7.4	2.2	0.5
E4D α -2-C1-IR	GFREGQFYDWFWVAQVT	43.1	11.6	5.0	2.3	0.4
E4D α -1-B4-IR	GFREGIFYEWFWVAQVT	45.3	6.6	2.9	2.3	0.4

FIG. 11-1

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Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	IR/IGFR
E4D α -4-H5-IR	GFREGNFYDWFVAQVT	--	--	--	--	--	--
E4D α -4-H5-IR	GFREGSFYEWFOAQVT	47.2	36.0	14.7	2.4	2.4	0.4
E4D α -1-B12-IR	GFREGNFYDWFVAQVT	47.6	33.4	13.8	2.4	2.4	0.4
E4D α -4-G2-IR	GFREGSFYDWFVAQVT	23.4	20.4	8.6	2.4	2.4	0.4
E4D α -3-F9-IR	GFREGDFYDWFVAQVT	36.2	15.6	6.3	2.5	2.5	0.4
E4D α -4-G6-IR	GFREGDFYQWFVAQVT	26.0	4.9	2.0	2.5	2.5	0.4
E4D α -4-H9-IR	GFREGGFYDWFVAQVT	47.8	24.8	9.5	2.6	2.6	0.4
E4D α -2-C10-IR	GFREGDFYDWFVAQVT	42.4	23.2	9.0	2.6	2.6	0.4
E4D α -1-B2-IR	GFREGVFYDWFVAQVT	39.4	18.7	7.2	2.6	2.6	0.4
E4D α -3-F12-IR	GFREGGFYEWFOAQVT	38.9	16.6	5.6	3.0	3.0	0.3
E4D α -2-D11-IR	GFREGSFYDWFVAQVT	40.2	11.1	3.3	3.4	3.4	0.3
E4D α -4-H2-IR	GFREGNFYEWFOAQVT	37.8	33.9	8.2	4.1	4.1	0.2
E4D β -4-A12-IR	GFREGKFYDWFVAQVT	41.1	8.3	28.7	0.3	0.3	3.5
E4D β -4-A10-IR	GFREGGFYEWFOAQVT	5.8	1.2	2.4	0.5	0.5	2.0
E4D β -4-E10-IR	GFREGVFYDWFVAQVT	9.6	1.2	2.2	0.5	0.5	1.8
E4D β -4-B11-IR	GFREGTFYDWFVAQVT	36.1	15.2	26.9	0.6	0.6	1.8
E4D β -4-C10-IR	GFREGGFYEWFOAQVT	27.8	13.3	23.7	0.6	0.6	1.8
E4D β -4-E8-IR	GFREGDFYEWFOAQVT	28.7	16.7	28.2	0.6	0.6	1.7
E4D β -4-G7-IR	GFREGHFYDWFVAQVT	30.9	14.7	24.7	0.6	0.6	1.7
E4D β -4-C8-IR	GFREGGFYDWFVAQVT	35.5	22.5	32.9	0.7	0.7	1.5
E4D β -4-A8-IR	GFREGSFYDWFVAQVT	31.2	14.5	22.2	0.7	0.7	1.5
E4D β -4-A9-IR	GFREGSFYDWFVAQVT	35.8	9.0	13.1	0.7	0.7	1.5
E4D β -4-G11-IR	GFREGTFYDWFVAQVT	28.9	9.7	13.6	0.7	0.7	1.4
E4D β -4-B9-IR	GFREGNFYEWFOAQVT	27.2	9.1	12.5	0.7	0.7	1.4
E4D β -4-F10-IR	GFREGSFYDWFVAQVT	7.7	1.5	2.1	0.7	0.7	1.4
E4D β -4-D12-IR	GFREGNFYDWFVAQVT	41.1	27.2	36.1	0.8	0.8	1.3
E4D β -4-B8-IR	GFREGDFYDWFVAQVT	35.9	27.0	35.2	0.8	0.8	1.3
E4D β -4-G10-IR	GFREGAFYDWFVAQVT	38.5	25.5	33.7	0.8	0.8	1.3
E4D β -4-D9-IR	GFREGSFYDWFVAQVT	34.1	19.3	25.7	0.8	0.8	1.3

FIG. 11-2

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	
E4Dβ-4-F8-IR	GFREGNFDWFVAQVT	--	--	--	--	--	--
E4Dβ-4-E12-IR	GFREGSFYDWFFAAQVT	39.3	35.6	44.4	0.8	1.2	1.2
E4Dβ-4-H12-IR	GFREGSFYEWFDAQVT	40.2	27.8	33.4	0.8	1.2	1.2
E4Dβ-4-C9-IR	GFREGAFYDWFEEAQVT	41.2	27.1	32.3	0.8	1.2	1.2
E4D□-4-H9-IR	GFREGQFYDWFFAAQVT	38.0	22.5	27.6	0.8	1.2	1.2
E4D□-4-G9-IR	GFREGNFDWFFAAQVT	38.7	33.3	36.6	0.9	1.1	1.1
E4Dβ-4-F12-IR	GFREGDFYDWFFAAQVT	10.9	4.9	5.6	0.9	1.1	1.1
E4Dβ-4-F9-IR	GFREGSFYEWFEAQVT	14.8	5.9	6.1	1.0	1.0	1.0
E4Dβ-4-F7-IR	GFREGGFYDWFLLAQVT	39.3	31.3	28.3	1.1	0.9	0.9
E4Dβ-4-B7-IR	GFREGGFYAWFEAQVT	31.0	22.2	19.5	1.1	0.9	0.9
	GFREGGFYENF?AQVT	--	--	--	--	--	--

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FIG. 11-3

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Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
E4D-2-E7-IGFR	GFREGNFYDWFVAQVT	--	--	--	--	--
E4D-2-C11-IGFR	GFREGDFYDWFRAQVT	20.8	22.8	--	--	--
E4D-2-B1-IGFR	GFREGSFYDWFVAQVT	21.5	22.6	--	--	--
E4D-2-D10-IGFR	GFREGDFYDWFQAQVT	22.0	22.5	--	--	--
E4D-2-A9-IGFR	GFREGGFYDWFQAQVT	20.6	22.1	--	--	--
E4D-2-E5-IGFR	GFREGDFYDWFVAQVT	17.4	21.5	--	--	--
E4D-2-H9-IGFR	GFREGDFYDWFQAQVT	24.2	21.2	--	--	--
E4D-1B-C4-IGFR	GFREGGFYDWFVAQVT	19.1	20.7	--	--	--
E4D-2-E10-IGFR	GFREGDFYDWFQAQVT	24.3	20.5	--	--	--
E4D-2-F4-IGFR	GFREGNFYDWFQAQVT	21.0	20.5	--	--	--
E4D-2-C10-IGFR	GFREGNFYDWFQAQVT	25.0	20.2	--	--	--
E4D-3-D8-IGFR	GFREGHFYDWFQAQVT	22.8	20.1	--	--	--
E4D-3-F9-IGFR	GFREGQFYDWFQAQVT	21.1	19.8	--	--	--
E4D-1B-E5-IGFR	GFREGSFYDWFQAQVT	22.6	19.7	--	--	--
E4D-2-F3-IGFR	GFREGDFYDWFQAQVT	24.2	18.8	--	--	--
E4D-3-D5-IGFR	GFREGHFYDWFVAQVT	23.6	18.0	--	--	--
E4D-3-G10-IGFR	GFREGQFYDWFQAQVT	22.2	18.0	--	--	--
E4D-2-F6-IGFR	GFREGQFYDWFQAQVT	22.1	17.6	--	--	--
E4D-2-F7-IGFR	GFREGQFYDWFQAQVT	24.6	17.5	--	--	--
E4D-3-B7-IGFR	GFREGDFYDWFQAQVT	19.0	17.5	--	--	--
E4D-1B-C12-IGFR	GFREGNFYDWFVAQVT	23.0	16.4	--	--	--
E4D-3-B1-IGFR	GFREGSFYDWFQAQVT	23.0	16.1	--	--	--
E4D-2-E2-IGFR	GFREGHFYDWFQAQVT	21.6	16.0	--	--	--
E4D-2-D1-IGFR	GFREGDFYDWFQAQVT	21.9	14.1	--	--	--
E4D-1-D4-IGFR	GFREGHFYDWFQAQVT	24.5	13.2	--	--	--
E4D-1B-A10-IGFR	GFREGYFYDWFKAQVT	18.9	12.4	--	--	--
E4D-1B-A3-IGFR	GFREGHFYDWFQAQVT	23.9	10.8	--	--	--
E4D-1-B5-IGFR	GFREGDFYDWFQAQVT	22.2	10.8	--	--	--
	GFREGTFYDWFVAQVT	19.0	10.8	--	--	--

FIG. 1J-1

Clone		Sequence	Ratios over Background			Comparisons	
Design			E-Tag	IGF _s R	IR	IGFR/IR	IR/IGFR
E4D-1B-B8-IGFR		GFREGNFDWFFVAQVT	--	--	--	--	--
E4D-1-G7-IGFR		GFREGDYGGWFEAQVT	23.8	10.7	--	--	--
E4D-1B-A11-IGFR		GFREGDFYAWFMAQVT	14.3	10.5	--	--	--
E4D-1-C3-IGFR		GFREGNFYEWFLAQVT	24.0	10.0	--	--	--
E4D-2-H1-IGFR		GFREGSFYDWFDAQVT	15.8	9.3	--	--	--
E4D-1-C2-IGFR		GFREGNFYDQFVAQVT	19.6	4.9	--	--	--
E4D-1B-A12-IGFR		GFREGHFYEWFAAQVT	11.5	4.5	--	--	--
E4D-1B-A1-IGFR		GFREGNFYEWFVAQVT	18.4	3.5	--	--	--
E4D-2-A3-IGFR		GFREGKFYDWFVAQVT	22.5	2.9	--	--	--
		GFREGMFDVQLLAQVT	22.7	2.1	--	--	--

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FIG. 1J-2

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
Parental	XXXXXXXXXXXXXXXXXXXX	--	--	--	--
H2CA-4-F11-IR	VTFTSAVFHENFYDFVFRQVSSXXXXXX	29.8	17.5	16.3	1.1 0.9
H2CA-4-E10-IR	TYKARFLHENFYDFNFRQVSQYFGRV	37.7	2.2	18.1	0.1 8.2
H2CA-4-G3-IR	QRSLHQFYDFVFGVSPLGAGG	31.2	4.4	18.8	0.2 4.3
H2CA-3-A11-IR	GGKVNFEHEDFYGFVQFSGVGS DR	36.1	13.4	25.7	0.5 1.9
H2CA-4-F8-IR	LVGDAPFHEDFYDFARQVFGCCQEQ	35.6	12.1	22.0	0.5 1.8
H2CA-4-G4-IR	TGAEVSFHENFYDFDRQYSSWLDRD	36.0	21.1	33.5	0.6 1.6
H2CA-4-F4-IR	QPHSSRLHESFYDFDRQVPWYALDR	37.1	23.3	34.3	0.7 1.5
H2CA-4-H10-IR	SPALAAVHEQFYDFVFRQVSGLDWGY	39.8	25.0	35.6	0.7 1.4
H2CA-4-F1-IR	QPKDGTLHENFYDFVFRQVSSSGWVG	33.5	5.1	6.6	0.8 1.3
H2CA-3-D5-IR	RGRLLQLHEDFYDFLFRQVSGMGGS	36.1	19.6	25.1	0.8 1.3
H2CA-4-E11-IR	QRGAPKSDENFYDFVFRQVLRFGEND	39.3	24.3	31.9	0.8 1.3
H2CA-3-B6-IR	AARTSLFHEDFYDFDRQVRQEGMWG	8.2	2.6	3.2	0.8 1.2
H2CA-3-A9-IR	GTSNHSLHENFYDFVFRQLSSVQSSG	35.9	9.9	12.1	0.8 1.2
H2CA-4-H5-IR	VSHVHLFHENFYDFVFRQLAAEGFSG	37.3	30.1	36.2	0.8 1.2
H2CA-3-C9-IR	GRQDSGLHEHFYDFSRQVQGEVALG	38.6	35.4	37.3	1.0 1.1
H2CA-3-A10-IR	SNDERQFHETFYDFVFRQVSADGADR	29.3	5.1	5.6	0.9 1.1
H2CA-3-A3-IR	LSTEQRFEHEFYDFVHVQVSTSGGT	37.2	16.9	19.1	0.9 1.1
H2CA-4-G8-IR	SLSREQFHENFYDFARQVSELEGVV	29.2	28.6	32.2	0.9 1.1
H2CA-4-G9-IR	IPGRRSLHENFYDFVFRQVSPGGGSA	32.4	29.1	31.6	0.9 1.1
H2CA-4-G10-IR	TQKAQSLDEKFYDFVFRQVSGGLTG	36.1	34.4	36.4	0.9 1.1
H2CA-4-H7-IR	VSQLSDFHENFYGFARQIAGQAEWT	34.2	35.5	37.7	0.9 1.1
H2CA-4-F9-IR	NGTSQALHQNFYDFWFAQQISGSEPGP	37.0	36.0	40.0	0.9 1.1
H2CA-4-F7-IR	VGQSVTFHGDFYDFDRQLSGSQEFG	37.5	36.7	39.5	0.9 1.1
H2CA-3-D10-IR	TIDHHPLEHQFYDFARQVSDLES LG	37.7	37.6	39.9	0.9 1.1
H2CA-3-B1-IR	PNVGAFHENFYDFIRQVSIIEEKAG	18.7	3.6	3.5	1.0 1.0
H2CA-3-A5-IR	SRGSGVFHESFYDFDRQVSEW IQFG	26.5	21.4	21.5	1.0 1.0
H2CA-4-F10-IR	QPVSGSVHERFYDFVFRQVSGSAGGG	32.9	22.9	22.4	1.0 1.0
	ASQLPPVYENFYDFDRQVSLDAQRE	26.6	27.7	28.5	1.0 1.0

FIG. 1K-1

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag --	IGFsR --	IR --	IGFR/IR --
H2CA-3-D9-IR	XXXXXXXXHNFYDFVFRQVXXXXXXXX	36.6	30.6	30.9	1.0
H2CA-3-C2-IR	VSGRGAFHNFYDFVFRQVFRDEQDT	38.3	30.7	31.0	1.0
H2CA-4-G1-IR	ARPPTVHNFYDFVFRQVSETWRQD	34.1	30.7	30.4	1.0
H2CA-4-E2-IR	QGGDRLFHNFYDFVFRQVSSDSTGE	33.9	31.0	31.8	1.0
H2CA-4-H9-IR	QHIAAGLHNFYDFVFRQVSGNVPA	38.9	31.1	31.4	1.0
H2CA-3-D2-IR	QPNDDLHNFYDFVFRQVSNVADGG	33.0	31.1	29.8	1.0
H2CA-3-B3-IR	PVEFTVYHNFYDFVFRQVSDGLGQF	35.3	31.4	30.0	1.0
H2CA-4-G11-IR	FCVQASIHNFYDFVFRQVAENQVFS	37.9	31.9	31.0	1.0
H2CA-4-F2-IR	GRPRGSFHNFYDFVFRQVSGDGAGT	38.1	32.0	31.9	1.0
H2CA-3-C5-IR	IVGASLCHESFYDFVFRQVTLQSQG	36.9	32.3	31.6	1.0
H2CA-3-B2-IR	IGLRQMTHNFYDFVFRQVSKAAGDG	36.6	32.7	32.5	1.0
H2CA-3-B11-IR	LGGATIEGHNFYDFVFRQVSLDVGE	35.5	32.8	33.3	1.0
H2CA-4-G2-IR	LNALQQLHNFYDFVFRQVSAATPPGG	35.9	33.0	33.4	1.0
H2CA-3-A4-IR	VGNCDTFPNFYDFVFRQVSELGGMN	33.3	33.0	32.9	1.0
H2CA-4-H3-IR	FSQDGNFHNFYDFVFRQVSLVGAGT	39.5	33.7	33.7	1.0
H2CA-4-G5-IR	PAGNRALHESFYDFVFRQVSEFQLGA	35.3	34.0	35.6	1.0
H2CA-4-E8-IR	DRLRARFNHNFYDFVFRQVSGGSMF	35.7	34.7	34.9	1.0
H2CA-4-G6-IR	VLGVAQFHDKFYDFVFRQVSQLSAG	36.2	35.0	33.5	1.0
H2CA-3-B7-IR	GVVGGAFHEQFYDFVFRQVSAAFKGD	37.6	36.5	35.3	1.0
H2CA-3-B4-IR	DESEMRLHEQFYDFVFRQVSLGGSA	39.8	36.5	35.1	1.0
H2CA-3-C7-IR	EGGGVAIHNFYDFVFRQVSLQGWSD	40.2	36.7	35.9	1.0
H2CA-4-E5-IR	SRIVSRFHNFYDFVFRQVSGDAPVQ	37.3	37.0	36.3	1.0
H2CA-4-E7-IR	IPAGAQLHNFYDFVFRQVSGEDGGA	39.7	37.6	37.6	1.0
H2CA-3-B9-IR	GSSAAGFDEQFYDFVFRQVSEAFRDG	39.4	37.7	37.6	1.0
H2CA-4-F5-IR	RLALRTFHQDFYDFVFRQVAAEDTDP	38.8	38.0	37.8	1.0
H2CA-3-B10-IR	QGSFAVLHNFYDFVFRQVSGVEGLA	41.9	38.9	38.0	1.0
H2CA-3-A12-IR	QGNMSALHNFYDFVFRQVSEADRVD	37.8	7.3	6.3	0.9
H2CA-3-A8-IR	VAYPALLHEQFYDFVFRQVSAVAGTT	36.8	22.5	19.2	1.2
	PDTINSQHKNFYDFVFRQVSGVGTSS				

FIG. 1K-2

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGF β R	IR	IGFR/IR	IR/IGFR	
H2CA-4-G7-IR	XXXXXXXXFHENFYDWFVRQVSXXXXXX	--	--	--	--	--	21/163
H2CA-3-C6-IR	RAGGVGLHDNFYDWFVRQVSGGDSGP	35.9	34.7	23.7	1.5	0.7	
H2CA-3-B8-IR	ADCYVQLHENFYDWFRRQVCNLQEGM	38.7	37.6	28.2	1.3	0.7	
	RQGHAGFHDNFYDWFVRQVSGSTPOV	37.8	19.6	9.9	2.0	0.5	

FIG. 1K-4

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Clone Design Parental	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
	XXXXXXXXXXXXXXXXXXXX VTFTSAVFHNFYDFWVFRQVSXXXXXX	--	--	--	--
H2CA-4-G9-IGFR	GIISQSCPESFYDNFAGQVSDPWWCW	29.8	17.5	16.3	1.1 0.9
H2CA-4-H6-IGFR	VGRASGFPENFYDNFGRQLSLQSGEQ	8.6	9.5	0.6	16.0 0.1
H2CA-4-F-IGFR5	VGYQQGQDENFYDNFIRQVSGRLGVQ	4.9	10.5	0.7	14.6 0.1
H2CA-4-H8-IGFR	SACQFDCHENFYDNFARQVSGGAAYG	5.5	9.7	0.8	12.3 0.1
H2CA-4-F11-IGFR	SAAQLFFQESFYDNFRLRQVAESSQPN	5.6	9.2	1.0	9.4 0.1
H2CA-4-F6-IGFR	AVRATRFDEAFYDNFVRQISDQGQNK	3.5	6.8	1.0	6.7 0.1
H2CA-4-F10-IGFR	VNQSISIENFYDNFVRQVSHQRGVR	3.9	7.3	1.1	6.4 0.2
H2CA-1-A3-IGFR	APDPSDFQEIFYDNFVRQVSRMPGGG	4.9	5.7	1.0	5.9 0.2
H2CA-3-C8-IGFR	SSCDGAGHESFYDNFVRQVSGCRSV	7.7	3.8	0.8	5.1 0.2
H2CA-2-B9-IGFR	RAGSSDFHEDFYDNFVRQVSLSLKQK	15.1	5.6	1.2	4.8 0.2
H2CA-4-H4-IGFR	QAVQPGFHEEFYDNFVRQVSTGVGGG	9.3	7.0	1.7	4.2 0.2
H2CA-4-F7-IGFR	SSIIGGFHENFYDNFWSRQLSQSPPLK	3.9	4.1	1.0	4.2 0.2
H2CA-3-D6-IGFR	QSPVGSSHEDFYDNFFFRQVAQSGAHQ	1.5	3.2	0.8	4.1 0.2
H2CA-3-D8-IGFR	NYRRQVFNGNFYDNFDRQVFSLVTPG	8.3	9.0	2.2	4.0 0.3
H2CA-4-G11-IGFR	TLDGGSFEEQFYDNFVRQLSYRTNPD	10.9	7.2	1.8	4.0 0.3
H2CA-4-F1-IGFR	FYVQQWGHENFYDNFDRQVSGSGGAG	10.8	9.5	2.5	3.9 0.3
H2CA-3-D7-IGFR	LRRQAPVEENFYDNFVRQVSGDRVGG	5.8	3.5	0.9	3.8 0.3
H2CA-1-A7-IGFR	RCGRELYHSTFYDNFDRQVAGRTCPS	13.3	3.0	0.8	3.7 0.3
H2CA-2-B4-IGFR	CCLLCRFQQNFYDNFVCQGISRLRPL	8.0	2.2	0.6	3.7 0.3
H2CA-2-B3-IGFR	PPLASDLVDQFYGNFVQVSPPPGRGG	3.5	4.1	1.1	3.6 0.3
H2CA-2-B2-IGFR	GAPVDQLHEDFYDNFVRQVQAATG	7.7	3.8	1.0	3.6 0.3
H2CA-3-D4-IGFR	RSASGSLPEQFYDNFVRQVSLSGTDK	4.1	3.4	1.0	3.5 0.3
H2CA-4-F2-IGFR	SRVTTVFHENFYDNFVRQLSDSAISG	17.6	13.8	4.1	3.4 0.3
H2CA-3-D11-IGFR	DERGGKFREDFYDNFVRQVSESRFGQ	9.3	12.8	4.2	3.0 0.3
H2CA-4-H9-IGFR	RGAVAGFHDQFYDNFDRQVSRVHKFG	12.2	6.9	2.3	3.0 0.3
H2CA-2-B11-IGFR	AICDAGFHEHFYDNFALQVSDCGRQS	8.7	5.6	1.9	3.0 0.3
H2CA-3-E8-IGFR	IGYQEPFQQNFYDNFVRQVSGAENAG	11.9	4.6	1.6	3.0 0.3
		13.2	6.3	2.2	2.9 0.3

FIG. 1L-1

Clone Design	Sequence	Ratios over Background				Comparisons	
		E-Tag	IGF ₃ R	IR	IGFR/IR	IR/IGFR	
	XXXXXXXXFHENFYDWFVVRQVSXXXXXXXX	--	--	--	--	--	--
H2CA-3-E6-IGFR	WRGHGTFHEDFYDWFVVRQVSGSGSST	15.7	8.7	3.1	2.8	0.4	
H2CA-4-F4-IGFR	GGRVGVLFHENFYDWFDRQVSLRGADG	11.5	7.4	3.0	2.5	0.4	
H2CA-3-D10-IGFR	CNLTAGFHEQFYHWFQVCGDAENA	9.4	6.8	2.9	2.3	0.4	
H2CA-3-E1-IGFR	ERGEDMFHENFYDWFVRQISGRQGG	12.5	6.4	2.8	2.3	0.4	
H2CA-2-B6-IGFR	TNQVGIFYDSFYGWVVRQIQYGVDSG	18.0	6.2	2.7	2.3	0.4	
H2CA-3-E11-IGFR	HLADGQFHEKFYDWFVRQISSRCNDC	4.7	2.2	1.0	2.2	0.5	
H2CA-4-H2-IGFR	QTFGKSLHENFYDWFVVRQVSREEGSD	9.8	9.9	4.8	2.1	0.5	
H2CA-3-C11-IGFR	FRTLAAQHDSFYDWFDRQVSGAAGER	9.3	3.3	1.6	2.1	0.5	
H2CA-2-B8-IGFR	SASTHQFHENFYDWFVVRQVSGAQKIL	14.6	7.9	3.9	2.0	0.5	

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FIG. 1L-2

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
Parental	XXXXXXXXXXFFXXWFXXXXXX	--	--	--	--
H2CB α -3-B12-IR	VTFTSAVFHENFYDWFRQVS	29.8	17.5	1.1	0.9
H2CB α -3-D2-IR	QSDSGTVHDFYGFWRDT*A	26.0	1.3	0.1	16.0
H2CB α -3-D12-IR	WTDVDGFHSGFYRWFNQWER	20.6	1.7	0.1	7.0
H2CB α -3-H5-IR	VASGHVLHGQFYRWFDQFAL	24.6	2.1	0.1	6.7
H2CB α -3-B6-IR	QARVGNVHQQFYWFREVMQG	16.7	2.4	0.2	6.3
H2CB α -3-G11-IR	VGDFCVSHDCFYGFLRESMQ	31.4	2.5	0.2	5.6
H2CB α -3-A6-IR	SGSRPVFHEQFYWFVDQLG	22.7	1.4	0.2	4.7
H2CB α -3-B1-IR	QFSAGAFHGDFYGFWRALYNG	25.9	1.7	0.2	4.3
H2CB α -3-F8-IR	SRFDERLHHQFYWFVRVLNEP	33.4	6.0	0.2	4.3
H2CB α -3-E11-IR	DSVNSDLHRAFYGWFAEQWRA	23.0	4.8	0.2	4.1
H2CB α -3-G4-IR	GSVDREIHGPFYSWFSEQLWG	14.0	2.2	0.3	4.0
H2CB α -3-D3-IR	SAKTPVLHDGFMWFEAQSES	24.9	2.2	0.3	3.2
H2CB α -3-C1-IR	LVVGRRFHQSFYDWFFVAAAGG	23.6	2.6	0.3	3.1
H2CB α -3-C3-IR	IMWPCTFQDPFYCWFTQTEQGR	27.0	5.6	0.3	2.9
H2CB α -3-G3-IR	VVGPLDIHERFYGWFFHQQGA	23.3	1.1	0.4	2.8
H2CB α -3-E4-IR	VVPKAGFHEAFYWFRRQDRD	23.7	6.7	0.4	2.6
H2CB α -3-G5-IR	QSFVTSVHTRFYAWFASALEM	28.8	8.3	0.4	2.6
H2CB α -3-B11-IR	SRGLGLYHSGFYGWFFERQFNQ	26.7	7.0	0.4	2.5
H2CB α -3-A1-IR	GADTGAVHRRFYLWFFELSGG	28.0	8.6	0.4	2.3
H2CB α -3-H1-IR	PGNRPTFHAFFYRWFFREAQGS	31.3	11.3	0.5	2.2
H2CB α -3-F12-IR	VAVAWGLHESFYAWFENQFSD	27.2	10.6	0.4	2.2
H2CB α -3-H7-IR	GFNTGTFHDQFYWFWEAAGG	21.1	6.1	0.5	2.1
H2CB α -3-C12-IR	GDGLTAFHQGFYWFEDIQMYG	21.0	9.7	0.5	2.0
	VGVNRQFHTRFYAWFDEQLGG	26.0	12.7	0.5	1.9

FIG. 1M-1

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	IR/IGFR
	XXXXXXFHXXFYXWFXXXXXX	--	--	--	--	--	--
H2CB α -3-D11-IR	GPRGQRLHDAFYSWFDALRVN	27.8	13.0	24.8	0.5	1.9	1.9
H2CB α -3-H12-IR	LGTAVFHFLFYGWFERQLGG	27.4	7.2	12.4	0.6	1.7	1.7
H2CB α -3-A10-IR	LGGYCGFNCQFYRWFDNLADR	27.1	13.2	22.3	0.6	1.7	1.7
H2CB α -3-A5-IR	FSGWADYQSGFYQWFAEELAN	28.3	16.1	28.1	0.6	1.7	1.7
H2CB α -3-C4-IR	WGPFSVFDESFYRWFAQASDD	30.7	17.2	29.2	0.6	1.7	1.7
H2CB α -3-B8-IR	PRNEGLVHGLFYDWFQALSG	25.6	11.3	18.6	0.6	1.6	1.6
H2CB α -3-H11-IR	DEGGAPLDVMFYRWFEQAVRG	28.8	14.0	22.4	0.6	1.6	1.6
H2CB α -3-E10-IR	QSGNRGSHGAFYSWFRDVLAN	27.7	14.3	23.0	0.6	1.6	1.6
H2CB α -3-C2-IR	MRQRDGFNSSFYGWFAAALGE	28.4	17.0	26.7	0.6	1.6	1.6
H2CB α -3-F6-IR	SEERKKVHSQFYSWFDRQLLG	27.3	14.5	21.8	0.7	1.5	1.5
H2CB α -3-D4-IR	PSPNAPFHGGFYDWFDDWVQGS	29.0	18.9	27.1	0.7	1.4	1.4
H2CB α -3-A7-IR	FHRPGSFNTNFYQWFDQMNQ	29.1	19.4	26.9	0.7	1.4	1.4
H2CB α -3-H4-IR	SDDSSTLNGREFYTWFMQLLD	27.2	20.1	27.9	0.7	1.4	1.4
H2CB α -3-B7-IR	QRGGGFHEGFYSWFRSQSLL	28.6	18.0	23.6	0.8	1.3	1.3
H2CB α -3-F9-IR	SGSRPVFHEQFYEWFDQLGL	26.1	19.1	24.3	0.8	1.3	1.3
H2CB α -3-H6-IR	GGSSQAFHGAFYEWFSALRG	24.8	21.6	27.3	0.8	1.3	1.3
H2CB α -3-F5-IR	AFVSERVNQRFYDWFDRDQMS	29.4	22.0	27.8	0.8	1.3	1.3
H2CB α -3-A2-IR	VRHPTREHDEFYRWFTTEQLTT	30.7	22.5	29.1	0.8	1.3	1.3
H2CB α -3-F3-IR	ARLLNIFDRGFYNWFQRQLDE	16.3	6.7	9.0	0.7	1.3	1.3
H2CB α -3-G6-IR	PSLSSNLHESFYRWFDQLVST	24.9	21.0	24.4	0.9	1.2	1.2
H2CB α -3-G7-IR	FAFGLGFHQGFYDWFHQLLEG	24.4	18.7	23.0	0.8	1.2	1.2
H2CB α -3-C5-IR	VSATVMLHREFYDWFGLQLLD	26.4	21.2	25.4	0.8	1.2	1.2
H2CB α -3-G1-IR	GGVSGVLHDRFYSWFERQLAG	26.9	21.5	26.3	0.8	1.2	1.2
H2CB α -3-E3-IR	GLGIASFHEGFYSWFTAQLGA	24.2	17.2	19.3	0.9	1.1	1.1

FIG. 1M-2

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF _s R	IR	IGFR/IR IR/IGFR
	XXXXXXXXXXFYXWFXXXXXX	--	--	--	--
H2CB α -3-A9-IR	RVDAALNAGFYEWFRGVIQG	30.5	21.7	24.1	0.9 1.1
H2CB α -3-C11-IR	GGAGRSFHDAFYEFERQWAG	26.4	21.8	23.2	0.9 1.1
H2CB α -3-B4-IR	EGARQGFHARFYSWFAQQAL	30.9	22.0	24.3	0.9 1.1
H2CB α -3-F11-IR	VLLPGVVHGGFYDWFSRQLSS	24.5	22.5	23.9	0.9 1.1
H2CB α -3-G10-IR	GALSDRYNNVFYDWFRQLLG	28.3	23.6	27.1	0.9 1.1
H2CB α -3-D7-IR	PDSFMSLHQRFYSWFQAQVGT	31.4	23.6	25.3	0.9 1.1
H2CB α -3-E2-IR	RVYKANFHNEFYGWFRQLLG	26.8	24.0	25.7	0.9 1.1
H2CB α -3-B5-IR	HSGMRDVHARFYSWFSEQLSG	28.7	25.0	26.4	0.9 1.1
H2CB α -3-C7-IR	ARLLERFQDPFYEFWFTLMGD	30.0	25.2	28.7	0.9 1.1
H2CB α -3-G9-IR	RNSSGNFHDKFYNWFEAQLKG	27.8	25.2	26.7	0.9 1.1
H2CB α -3-A12-IR	GSMSPVFNDQFYGWFRDLVDE	28.0	26.4	28.7	0.9 1.1
H2CB α -3-C9-IR	SCTGRQFDGCFYAWFEDQLVG	32.1	28.7	31.9	0.9 1.1
H2CB α -3-B10-IR	GIAVQSLHDSFYRWFDNALGS	33.5	30.8	33.2	0.9 1.1
H2CB α -3-E1-IR	IGPPGSLHRGFYDWFAEQVEA	31.7	30.5	29.0	1.1 1.0
H2CB α -3-G12-IR	GAAGISFHRGFYDWFAAQVRD	29.1	31.4	29.8	1.1 1.0
H2CB α -3-F7-IR	GVDVTDHFKDFYSWFQRQLNG	23.2	20.7	20.3	1.0 1.0
H2CB α -3-G8-IR	WAGRAGIHGGFYEFWFRQLRG	22.8	20.9	20.4	1.0 1.0
H2CB α -3-C6-IR	LGQLAAPHHLGFYEFWFEAVAA	26.7	21.2	22.0	1.0 1.0
H2CB α -3-H9-IR	VHSVSRLNVGFYQWFQDQLSG	23.4	22.5	22.0	1.0 1.0
H2CB α -3-H8-IR	LGLMAIFDRGFYGFYEFQQLSG	23.5	23.4	23.2	1.0 1.0
H2CB α -3-F2-IR	VARGSSLHDDFYEFWFAQLRT	25.5	24.3	25.2	1.0 1.0
H2CB α -3-D5-IR	LGYGALNTQFYSWFADLVGS	26.7	24.5	25.6	1.0 1.0
H2CB α -3-D10-IR	EDSRRLRHEGFYGFYWRKQLGD	26.8	24.9	24.9	1.0 1.0
H2CB α -3-F10-IR	GRDNMKFHSGFYDWFTQQLAG	25.7	25.6	26.1	1.0 1.0

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FIG. 1M-3

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	XXXXXXXXXXXXXX	--	--	--	--	--
H2CB α -3-D6-IR	AGVMGGFHQEFYLVFERALSN	27.9	26.0	25.8	1.0	1.0
H2CB α -3-H3-IR	AGHVGQVYDGFYGFWEQLGA	27.0	26.9	26.2	1.0	1.0
H2CB α -3-F4-IR	FVQNI $\overline{\text{G}}$ FDYDFYGFVREVEK	31.2	27.2	27.7	1.0	1.0
H2CB α -3-E9-IR	PVGIGGLHRAFYQWFQSQVDA	31.6	27.7	28.2	1.0	1.0
H2CB α -3-H10-IR	GSRQ $\overline{\text{E}}$ ADHQAFYDWFNLVLGV	26.9	27.9	28.8	1.0	1.0
H2CB α -3-G2-IR	AGGRKPFHDDFYGWF $\overline{\text{R}}$ DQLAE	29.1	28.1	28.8	1.0	1.0
H2CB α -3-B2-IR	DLASHGFHDAFYNWFSVQLNS	29.4	28.1	28.2	1.0	1.0
H2CB α -3-E8-IR	GSNGGGVHGQFYAWFVEALSG	31.5	28.4	29.1	1.0	1.0
H2CB α -3-E5-IR	RGRASTFHDGFYGFWSQQLRF	33.0	28.7	28.9	1.0	1.0
H2CB α -3-E6-IR	SPARRVSHHDFYGFWEAKQLES	29.6	29.0	28.1	1.0	1.0
H2CB α -3-E7-IR	SSDVGA $\overline{\text{F}}$ HSAFYDWFKAQLSG	30.4	30.2	30.2	1.0	1.0
H2CB α -3-C8-IR	PTVHRAFD $\overline{\text{D}}$ LFYGFWEAKQVED	31.9	31.2	31.5	1.0	1.0
H2CB α -3-A4-IR	SSNTVGLDERFYAWFVDQLGA	32.2	31.9	32.6	1.0	1.0
H2CB α -3-D1-IR	PGAAEGFHS $\overline{\text{A}}$ FYDWFQAQAVSG	32.9	32.5	31.5	1.0	1.0
H2CB α -3-B9-IR	MRSEASFHVEFYSWFEEQLRS	33.2	33.8	33.3	1.0	1.0
H2CB α -3-D8-IR	VSRYG $\overline{\text{G}}$ QDGFYHWFSDLLKG	26.3	20.2	19.1	1.1	0.9
H2CB α -3-F1-IR	RPSSGGLHYGFYHWFVRVQEEM	28.8	28.0	26.4	1.1	0.9
H2CB α -3-A11-IR	SNIEEHFMQFYRWFS $\overline{\text{D}}$ ALGN	20.5	21.5	17.7	1.2	0.8
H2CB α -3-A3-IR	ANDCLGLHAGFYGF $\overline{\text{W}}$ FACQLGG	30.4	29.6	21.8	1.4	0.7

FIG. 1M-4

Clone Design	Sequence	Ratios over Background				Comparisons	
		E-Tag --	IGFsR --	IR --	IGFR/IR --	IR/IGFR --	--
H2CB β -3-E8-IR	XXXXXXXXXXXXXXXXXXXXX TGRLGLDEQFYWFRDALSG	15.9	1.9	11.8	0.2	6.1	6.1
H2CB β -4-F8-IR	VLTSNTLHQRFYSWFAAARRE	13.4	0.8	2.6	0.3	3.4	3.4
H2CB β -3-C4-IR	CVAQGGFQSSFYCWFAGLDID	21.1	1.3	4.0	0.3	3.1	3.1
H2CB β -3-D5-IR	NGQSSRFHTAFYDWFAAQLSG	14.0	3.3	10.2	0.3	3.1	3.1
H2CB β -3-E6-IR	SVPRGTVHDAFYQWFREVALG	5.7	0.7	2.1	0.3	3.1	3.1
H2CB β -4-G12-IR	GARGSTFHDQFYEFWFWVQLGD	6.8	1.8	5.4	0.3	3.1	3.1
H2CB β -4-F4-IR	PPGMNGFHTSFYSWFDQLGD	17.9	1.9	5.6	0.3	3.0	3.0
H2CB β -4-F11-IR	AVGTLGYHSGFYRWFERQLGG	15.0	1.7	4.8	0.3	2.9	2.9
H2CB β -3-E5-IR	ELQARGVHRNFYRWFEAQVSG	17.0	1.8	5.0	0.4	2.8	2.8
H2CB β -4-F2-IR	HRVARAFHEQFYDWFEKAVSG	15.9	1.3	3.4	0.4	2.6	2.6
H2CB β -4-G4-IR	GAMEPDYHRSFYQWFAAALGE	8.7	1.4	3.5	0.4	2.6	2.6
H2CB β -3-C8-IR	CPDRQSVDDRFYNWFADALAS	4.9	1.4	3.2	0.4	2.3	2.3
H2CB β -4-F10-IR	GGAQISFHERFYQWFLQEAAG	10.2	1.0	2.4	0.4	2.3	2.3
H2CB β -4-H4-IR	HKRGIVQHGAFAFWFDSLLSG	20.8	4.2	9.5	0.4	2.3	2.3
H2CB β -4-G6-IR	QASDNRS $\overline{\text{D}}$ GQFYLWFEKLLSS	14.5	5.6	8.5	0.7	1.5	1.5
H2CB β -4-H1-IR	DRGRMGVDEGFYNWFARQM $\overline{\text{Q}}$ E	17.0	10.1	13.2	0.8	1.3	1.3

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FIG. 1M-5

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGF _s R	IR	IGF _s R/IR	IR/IGF _s R
Parental	XXXXXXXXXXFYXWFXXXXX	--	--	--	--	--
H2CB-3-D2-IGFR	VTFTSAVFHENFYDWFVRQVS	29.8	17.5	16.3	1.1	0.9
H2CB-3-C12-IGFR	TASQECFDDGFYGFRAWRCT	22.9	18.6	11.8	1.6	0.6
H2CB-3-B11-IGFR	SLDWRWSEEPFYRWFORALAG	17.3	19.6	13.0	1.5	0.7
H2CB-4-E2-IGFR	CMSLSDCHRKFFYGFWSQGE	24.6	17.1	11.9	1.4	0.7
H2CB-3-A5-IGFR	LALCRRSPGSFYGFQAAVGC	22.4	21.0	16.5	1.3	0.8
H2CB-4-G12-IGFR	PRSATMSDGGFYWWFASQLGL	28.8	26.1	22.6	1.2	0.9
H2CB-3-B2-IGFR	LRRSSVFHDPPYE*ISRLVGG	23.7	23.8	19.4	1.2	0.8
H2CB-3-D1-IGFR	ARLQQQFHGGFYEWFRQAQVSP	23.0	19.9	16.4	1.2	0.8
H2CB-3-B6-IGFR	AQLDNLCHPEPFYSWFCAVTR	21.5	19.5	15.7	1.2	0.8
H2CB-4-F7-IGFR	WTCDTAFHQDFYQWFCDKLG	16.3	4.5	3.7	1.2	0.8
H2CB-4-G8-IGFR	GKEGFGLDLDRDFYWWFREQLGP	22.0	19.0	18.0	1.1	0.9
H2CB-3-D4-IGFR	GRAPSSFCDFYCWFERNQVQS	20.2	18.6	16.5	1.1	0.9
H2CB-3-D5-IGFR	DVEAETQHRLFYAWFLSQLGS	21.9	18.3	16.9	1.1	0.9
H2CB-4-E6-IGFR	ISVTAVFHDBGFYGFWEQVSK	21.4	17.9	16.4	1.1	0.9
H2CB-3-C2-IGFR	NSEHGRLDVFYGFWARVIQQ	19.6	15.8	14.8	1.1	0.9
H2CB-3-A6-IGFR	GPLGDGCQDGFYGFWMQCVST	18.8	12.2	10.8	1.1	0.9
H2CB-4-H12-IGFR	KRSAYNFHDPPFYDWFMRQLSG	26.8	29.0	28.1	1.0	1.0
H2CB-3-B10-IGFR	ASEPGGYLDPFYGFWEQLRA	23.9	28.3	28.1	1.0	1.0
H2CB-4-F11-IGFR	NRGDGGVHSGFYWNFRLQLSG	27.1	27.5	27.3	1.0	1.0
H2CB-4-G11-IGFR	ASKGSSLHNDFYGFWFAQQLAR	25.5	25.5	24.6	1.0	1.0
H2CB-4-E12-IGFR	ANVSMWIOVGFYDWFDAQLRQ	25.3	25.4	25.3	1.0	1.0
H2CB-4-G10-IGFR	RTSPGSLHDPFYDWFQQQLGG	27.8	24.9	24.7	1.0	1.0
H2CB-3-B9-IGFR	PGVMSSFHGGFYSWFREQLNG	25.1	24.6	24.2	1.0	1.0
H2CB-3-B7-IGFR	CLANSEHDHDSFYGFWFCQALGG	25.6	23.3	23.7	1.0	1.0
H2CB-4-H4-IGFR	GGSMGMHGSFYENFALQLRS	24.0	23.2	23.5	1.0	1.0
	RPQGGSIHAGFYQWFRDAVAG	23.5	23.1	23.8	1.0	1.0

FIG. 1N-1

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
H2CB-4-H10-IGFR	XXXXXXXXXXFYXWFXXXXXX	21.9	22.4	23.3	1.0
H2CB-4-H5-IGFR	GALSSLFDAAFYDWFNRQLG	22.3	22.3	21.6	1.0
H2CB-4-G7-IGFR	KVDLRGFHDGFYGFARQLAG	23.1	21.6	20.6	1.0
H2CB-4-F4-IGFR	CSGLQRCHDSFYSWFESVRE	21.3	20.9	21.3	1.0
H2CB-3-D8-IGFR	DSLGISFHEGFYDWFRRQLDM	20.0	20.5	21.6	1.0
H2CB-4-E4-IGFR	SGVFNGTFYDWFRIQLGE	21.6	20.5	21.2	1.0
H2CB-4-E5-IGFR	GYREMRSDLGFYQWFRDQLGL	22.0	19.9	20.9	1.0
H2CB-4-E8-IGFR	SVFMQHDHVGFYAWFRSLMEE	21.1	19.7	20.7	1.0
H2CB-3-D12-IGFR	FRHITEVDRSFYGFVEQLRG	26.6	17.3	16.8	1.0
H2CB-4-G9-IGFR	WAGGSDVDGSFYDWFQRLLAS	21.6	14.5	15.2	1.0
H2CB-3-C8-IGFR	GLQNVSFHSGFYQWFEVVRG	20.8	13.4	13.9	1.0
H2CB-3-A12-IGFR	SRVSDPYHVGFYQWFEVVRG	28.6	27.5	29.2	0.9
H2CB-3-B12-IGFR	MGGATFFHTGFYDWFQRLQH	27.8	25.2	27.1	0.9
H2CB-3-A9-IGFR	RPASRPFHSGFYQWFEVVRG	27.7	24.3	25.7	0.9
H2CB-3-A3-IGFR	GLAPGNFHEFYRWFOEQLTG	26.9	24.1	26.5	0.9
H2CB-3-B4-IGFR	TAAISDFNSLFYGFVEQLSS	25.8	23.8	25.3	0.9
H2CB-4-E7-IGFR	LDEDLPQHAGFYGWFAEALGV	24.6	21.6	24.0	0.9
H2CB-4-G6-IGFR	ASHKSAFDDNFYRWFSMQLRD	22.4	21.1	23.0	0.9
H2CB-4-E9-IGFR	HTGAGDLHGAFFYNWFLEQLGG	24.3	20.7	22.0	0.9
H2CB-4-H2-IGFR	RRGRDGFHGGFYDWFQRLSD	21.6	20.2	21.9	0.9
H2CB-3-A10-IGFR	GNFREAFHADFYSWFERQLQS	24.3	19.9	21.5	0.9
H2CB-3-C4-IGFR	RDTLPAFHQHFYQWFEKQVSA	23.1	19.2	22.0	0.9
H2CB-3-B5-IGFR	ERETAAFGQAFYQWFRDQIAG	24.2	18.8	20.7	0.9
H2CB-4-G4-IGFR	WGEKGGFYDWFYDQLGWEP SH	21.7	18.7	21.2	0.9
H2CB-3-D9-IGFR	SLVAADLHEGFYGFWRFSQLGG	24.4	18.6	20.0	0.9
H2CB-3-C3-IGFR	TSEVGDFAEFYSWFEIQLGR	20.3	18.4	21.1	0.9
H2CB-3-D3-IGFR	TGADGLLHARFYAWFEEQLRE	22.5	18.3	21.3	0.9
H2CB-4-F2-IGFR	RRSDSSLHRSFYDWFVSQLLN	18.0	16.8	18.3	0.9
	SESKYLLHSGFYGWFEAQLRG				

FIG. 1N-2

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFsR/IR/IGFR
H2CB-4-H1-IGFR	XXXXXXXXXXXXXX	18.3	15.3	16.5	0.9
H2CB-4-F9-IGFR	HGVI RADHTGFGYGFWSKQLSD	22.9	14.4	15.3	0.9
H2CB-4-E10-IGFR	LINA.VFRRGFYAWFEEQVSK	26.1	20.1	24.5	0.8
H2CB-4-F8-IGFR	LQRYIGFHDPPYDFWFSRALS	21.5	14.8	19.0	0.8
H2CB-3-A8-IGFR	MRTAELFHVGFYDFWFDQLMD	20.7	14.7	18.2	0.8
H2CB-4-F1-IGFR	WAPPDALHGQFYRWFORQLDQ	22.2	14.6	18.8	0.8
H2CB-3-C6-IGFR	AVHAATFHDDFYRWFEQVVG	15.7	7.8	10.2	0.8
H2CB-4-E11-IGFR	FDAVHGFDGGFYGWFKRELQR	26.1	17.6	24.1	0.7
H2CB-3-D6-IGFR	QAGMEFHGAFYNWFLQQLSG	21.6	13.0	18.8	0.7
H2CB-4-F3-IGFR	GRSVSRMNAEFYQWFGHQLAA	17.3	11.1	16.4	0.7
H2CB-3-A4-IGFR	AAVNSLFHDEFYLVFQDQLDG	27.4	11.0	14.8	0.7
H2CB-3-B1-IGFR	QLGMDWFHADFYEWFLAQLPS	20.0	11.0	15.2	0.7
H2CB-3-C5-IGFR	RLAGSGIHEGFGYGFVDQLLA	19.9	10.5	15.6	0.7
H2CB-4-F6-IGFR	GREIGGVHDGFYDFWRQQLSE	18.6	10.1	14.6	0.7
H2CB-3-B8-IGFR	VRSEQRFDSSFYQWFDLLMS	20.7	6.9	9.5	0.7
H2CB-3-C7-IGFR	QSPYGFHHDGFYRWFLQQTGM	16.2	1.8	2.5	0.7
H2CB-4-H7-IGFR	FQCGAAFHVDYRWFTCQEQF	21.8	14.1	22.7	0.6
H2CB-4-F5-IGFR	GAFGSEFHEQFYRWFEALSF	12.9	4.0	7.2	0.6
H2CB-4-G1-IGFR	EHTSYQIHRQFYEWFDRLGR	20.4	10.3	19.7	0.5
H2CB-3-D11-IGFR	SGTAAADLHSRFYGFALQARE	24.1	8.8	18.6	0.5
H2CB-3-D7-IGFR	EGFGVLFHGQFYRWFLQLDQ	22.1	6.5	13.6	0.5
H2CB-3-C10-IGFR	QQSAGHPHSSFYLVFSELLGA	21.7	5.1	10.4	0.5
H2CB-4-E3-IGFR	YLQRAGFHRSEFYGFWDQALRD	20.3	4.6	8.9	0.5
H2CB-3-C1-IGFR	MWLWATLHSDFSYSWFEQVVG	22.3	6.7	15.7	0.4
H2CB-4-G2-IGFR	GANALGFKDRFYEWFAAQLWD	19.9	3.3	10.7	0.3
H2CB-3-A11-IGFR	GSGLYVFHWGFYDWFEQQMG	23.9	2.5	7.7	0.3
H2CB-4-G5-IGFR	LDKGWGFDLQFYRWFEAATRA	19.3	2.5	7.9	0.3
H2CB-4-F12-IGFR	QSAVEFHADFYDWFLRLTTP	16.7	1.7	5.4	0.3
	DQRMGSFHGEFYRWFEETLLS				

FIG. 1N-3

Clone Design	Sequence X _n -Fy _n WF-X _n	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
		--	--	--	--
20E2A-3-B11-IR	GRFYGWFFQDAIDQLMPWGFD	24.6	1.4	23.6	0.1
20E2Bβ-3-E3-IR	IQGWEPFYGFDDVVAQMFE	23.0	0.9	15.3	0.1
rB6-3-F6-IR	RYGRWGLAQQFYDFDR	40.9	1.0	13.3	0.1
rB6-4-F9-IR	RRLGSLSTQFYNNWFAE	34.1	1.0	12.6	0.1
20E2Bα-3-A8-IR	ASAYTPFYQWFADVVSEYMQQ	35.4	7.4	34.4	0.2
A6L-4-F6-IR	PYRMEGTEKWNFYDFVAQLQ	28.9	4.1	18.1	0.2
20E2Bα-4-H9-IR	SAVHFQFYKWFNDLLPVPLSA	37.8	9.4	26.7	0.4
20E2Bα-3-B1-IR	VPVNKSFYRWFFQLVLGGDDW	41.8	12.9	36.8	0.4
20E2Bβ-4-F9-IR	QSPRASFYGWFFDDVLRAGVV	25.9	4.2	10.1	0.4
20E2Bβ-3-E9-IR	TGFYEWFYEQHLHSRMLPNPLD	27.0	7.7	17.2	0.5
20E2Bβ-3-E10-IR	RRGVGGFYGWFSQQQLQGMGVA	22.2	2.6	5.5	0.5
20E2Bα-3-C12-IR	SSQDRRFYRWFEQAIVGGRDG	39.0	6.7	12.0	0.6
20E2Bβ-3-C12-IR	TRGQLGFYNNWFQQALSTSGMG	20.2	2.2	3.8	0.6
20E2Bβ-3-E7-IR	CADLNAFYQWFCGVLDGRGSDH	9.2	1.2	1.9	0.6
20E2Bβ-3-E11-IR	TLIQDQFYWWSFSDLLSAEPGD	20.7	1.3	2.1	0.6
20E2Bα-3-B11-IR	IDQLDAFYRWFDGVMGLMGDP	36.0	20.7	32.8	0.6
NNKH-4-G2-IR	RGGGTFYEWFE ² SALRKHGAG	10.8	6.3	8.9	0.7
20E2Bα-3-A7-IR	RGLDQDFYRWFFQNLVGVEYDR	19.0	4.2	5.5	0.8
20E2Bα-4-G12-IR	MQHGRGFYGFARVLEQDRGW	37.0	22.3	29.5	0.8
20E2Bα-3-C11-IR	ERLHLRFYEWFD ² VIGQDGS	37.3	26.8	34.8	0.8
20E2Bα-3-C10-IR	MHVQSDFYHWFQSLLGQGGPD	37.7	24.8	30.5	0.8

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FIG. 10-1

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Clone Design	Sequence X_n -FyxWF- X_n	Ratios over Background			Comparisons		
		E-Tag --	IGFsR --	IR --	IGFR/IR --	IR/IGFR --	--
20E2B α -3-D7-IR	TMGTQGFYRWFQNVVKEHLSG	35.4	26.9	31.3	0.9	1.2	
20E2B α -3-A12-IR	ITHNRGFYSWFLDVVQGGAGA	31.7	22.0	23.3	0.9	1.1	
20E2B α -3-D10-IR	VRRDAGFYQWFADILTQDFFE	32.7	27.3	29.1	0.9	1.1	
20E2B α -4-G7-IR	MLQDEFYNWFRGIMLNDGQD	34.2	29.0	30.7	0.9	1.1	
20E2B α -4-F5-IR	GIRSSGFYQWFDRLVLAGVGDG	33.8	32.1	34.0	0.9	1.1	
20E2B α -3-C9-IR	ANLSQFYSWFASVTGEASPS	39.4	33.2	35.5	0.9	1.1	
20E2B α -3-A4-IR	QSPRASFGWFDLVLRAAGVV	38.2	31.6	35.9	0.9	1.1	
20E2B α -4-E12-IR	MQRNQGFSWFDDLVSSTVGV	36.0	30.8	29.7	1.0	1.0	
20E2B α -4-E11-IR	ASGFDPPFYAWFLEQLRVANGS	35.1	31.2	30.7	1.0	1.0	
20E2B α -4-E8-IR	SGTPYGFYRWFQSALASATSG	36.1	30.5	30.7	1.0	1.0	
20E2B α -4-H10-IR	QGVGGFYWFDRAMGDVRPW	38.9	30.6	30.7	1.0	1.0	
20E2B α -4-F6-IR	DNMSGGFYRWFQVVADSGGD	34.9	33.2	32.0	1.0	1.0	
20E2B α -4-G4-IR	RGTDDTFYGWFDQLLQWCDD	34.1	33.7	32.2	1.0	1.0	
20E2B α -4-F8-IR	TVDHTQFYDWFSRVLGESGSA	37.7	32.0	32.7	1.0	1.0	
20E2B α -4-G5-IR	GRQDREFYWFELQAGGMDGD	34.9	33.9	33.4	1.0	1.0	
20E2B α -3-B10-IR	RLLLGGFYWFQVLKETKEV	38.2	34.9	33.6	1.0	1.0	
20E2B α -3-C7-IR	GVLSTGFYWFALQLHGLAAG	37.6	34.2	34.8	1.0	1.0	
20E2B α -3-C5-IR	PAVGQSFYGWFEAVLRGSKAG	40.4	36.0	35.6	1.0	1.0	
20E2B α -3-B9-IR	SNGISGFYWFQAAQVQTSDFQ	39.6	35.8	37.1	1.0	1.0	
A6L-4-F11-IR	LLGLSQAAYANFYDWVFSQLA	33.1	4.6	4.6	1.0	1.0	
20E2B α -3-C2-IR	VPNSWMFYNWFQIEGSEGE	44.1	40.0	38.1	1.0	1.0	
20E2B α -3-B2-IR	ARRADGFYDWFEQVSGSAVQ	43.1	40.1	39.0	1.0	1.0	
20E2B α -4-G2-IR	GVVEGTFYWFDRLLGGVQGD	34.1	33.6	29.8	1.1	0.9	
20E2B α -4-H6-IR	SHLTDPFYQWFVDQLRAGVRG	39.4	36.0	31.9	1.1	0.9	

FIG. 10-2

Clone Design	Sequence X _n -FyxWF-X _n	Ratios over Background			Comparisons	
		E-Tag	IGF ₃ R	IR	IGFR/IR	IR/IGFR
20E2Bα-4-H5-IR	RSNDDAFYRWFSNIIQVDGGG	38.7	35.1	32.3	1.1	0.9
20E2Bα-4-G3-IR	DSDGAQFYIWFEQ ¹ LSAGWD	35.5	36.1	32.7	1.1	0.9
20E2Bα-4-H4-IR	PGLHRAFYQWF ² AEAVRSANKE	38.8	37.9	35.0	1.1	0.9
20E2Bα-3-C1-IR	SLGQGGFYDWF ³ ASQVGGADI	43.7	42.1	39.0	1.1	0.9
20E2Bα-4-E6-IR	CGQTQSFYQWFCEVMRVESGD	38.0	34.3	29.7	1.2	0.9
H5-3-D5-IR	IVVPGDTQGVNFYDWFVKQLQ	43.8	21.8	18.2	1.2	0.8
JBA5-3-D9-IR	RDVSMGSASTNFYDWFVQQLG	38.3	29.8	25.3	1.2	0.8
20E2Bβ-4-G6-IR	SQAGSAFYAWFDQ ⁴ VLRTVHSA	22.4	6.2	1.9	3.3	0.3
20E2Bβ-4-H10-IR	SNGISGFYEWFAAQVQ ⁵ TSDFQ	23.5	32.2	9.7	3.3	0.3
rB6-4-G8-IR	RRDRGGLDVFFYQWFMD	--	--	--	--	--

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FIG. 10-3

Ratios over Background		Comparisons	
E-Tag	IGF ₃ R	IGFR/IR	IR/IGFR
--	--	--	--
39.1	1.8	27.7	15.4

Sequence
XXXXXXXXXXXXXXXXXXXX
HLCVLEELFWGASLFGYCSG

Clone
Design
R20β-4-F8-IR

FIG. 2A

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Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGF ₃ R	IR	IGFR/IR	IR/IGFR	IR/IGFR
F815-4-H9-IR	HLCVLEELFWGASLFGYCSG	--	--	--	--	--	--
F815-3-B1-IR	PLCVLEELFWSTPLFGQCSY	34.9	0.9	37.6	<0.1	<0.1	40.8
F815-3-D1-IR	HLCVLEELFWGASLFAQC VG	31.7	0.9	35.8	<0.1	<0.1	39.3
F815-3-D4-IR	DLCVLEELFWGASRFGQCSG	30.4	0.9	33.5	<0.1	<0.1	38.9
F815-3-C5-IR	HLCVLEELFWGASLFGQCAG	31.5	0.9	33.6	<0.1	<0.1	38.8
F815-4-H3-IR	HLCVVEELFWGASLFGQCSG	31.1	0.8	31.2	<0.1	<0.1	38.5
F815-3-A5-IR	NLCDLELVFWGASLFRQCSG	33.7	1.0	37.2	<0.1	<0.1	38.4
F815-3-D7-IR	PLCVLEEQFWGASLFGQCSG	37.4	1.1	40.9	<0.1	<0.1	38.3
F815-3-A1-IR	QLCVLEELFWGASEFGQCSG	33.6	0.9	34.3	<0.1	<0.1	38.3
F815-4-H4-IR	HLCVLEELFWGASLFGQCSG	29.8	0.9	34.8	<0.1	<0.1	38.0
F815-3-A3-IR	PLCVLEELFWGESLFGQCSG	31.1	0.9	32.7	<0.1	<0.1	38.0
F815-3-B3-IR	HLCVLEELFWGASRFGQCSG	32.8	1.0	39.1	<0.1	<0.1	37.9
F815-3-A4-IR	KLCVLEELFWGASLFGQCSG	33.7	1.0	37.5	<0.1	<0.1	37.5
F815-3-D2-IR	YLCVLEELSWGASLFGQCSG	32.5	1.0	36.9	<0.1	<0.1	37.5
F815-3-C4-IR	HLCVLEELLWGASLFAQC SG	31.9	0.9	34.1	<0.1	<0.1	37.4
F815-3-B4-IR	QLCVLEQLFWGESLFGQCSG	31.6	0.8	31.8	<0.1	<0.1	37.4
F815-3-C1-IR	HLCVLEELFWGGNLFSQCSG	33.8	1.0	36.7	<0.1	<0.1	37.3
F815-4-G9-IR	HLCVLEELFWGASLYGQCSG	29.0	0.9	35.0	<0.1	<0.1	37.3
F815-4-G6-IR	SLCALEEQFWGAALFGYCSG	36.5	1.0	38.9	<0.1	<0.1	37.1
F815-3-A8-IR	HLCVLEEQFWGASLFDGCAG	34.9	1.0	36.4	<0.1	<0.1	37.0
F815-4-G5-IR	QLCVLEELFWGASLFGQCSG	34.7	1.1	39.3	<0.1	<0.1	36.9
F815-3-B5-IR	PLCVLEELFWGAALFGQCSG	26.5	1.0	35.1	<0.1	<0.1	36.8
F815-4-F4-IR	HLCVLEELFWGASLFGQCTG	33.2	0.9	34.1	<0.1	<0.1	36.8
F815-3-A2-IR	PLCVLEELFWGGS LFGQCSG	28.6	0.8	30.0	<0.1	<0.1	36.7
F815-3-B6-IR	QLCVLEELVWGASLFGQCSG	32.5	1.0	36.6	<0.1	<0.1	36.6
F815-4-H7-IR	HLCVVEELIWGASLFGQCSR	31.6	0.9	32.9	<0.1	<0.1	36.5
F815-4-H8-IR	DLCVLEELFWGASLFGQCAG	33.7	1.0	37.6	<0.1	<0.1	36.4
F815-4-G7-IR	QLCVLEERFWGASLFGQCSG	35.8	1.0	37.0	<0.1	<0.1	36.4
	NLCVLEELFWGAALFGQCSG	33.7	1.0	35.8	<0.1	<0.1	36.3

FIG. 2B-1

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
F815-3-A6-IR	HLCVLEELFWGASLFGYCSCG	34.6	1.1	<0.1	36.2
F815-3-D3-IR	QLCVLEELFWGSSLFGQCSCG	33.8	1.0	<0.1	36.2
F815-3-B12-IR	DLCVVEELFWGKSLFGQCSCG	33.2	1.0	<0.1	36.2
F815-4-G10-IR	DLCVLEELFWGSSLFGQCSCG	35.4	1.0	<0.1	36.1
F815-4-E3-IR	YLCVLEEQFWGASLFRQCFCG	32.4	1.0	<0.1	36.1
F815-4-E6-IR	HLCVLEELLWGSSLFGQCSCG	33.2	1.0	<0.1	36.1
F815-4-F1-IR	PLCVLEELFWGASLFGQCSD	29.4	0.9	<0.1	36.0
F815-4-G8-IR	HLCVLEELFWGSSLFAQCSCG	36.8	1.1	<0.1	35.9
F815-4-H12-IR	PLCAIEELFWGAALFGQCSCG	30.5	0.9	<0.1	35.9
F815-4-G3-IR	HLCVLEEQFWGASLFGDCSCG	31.4	1.0	<0.1	35.7
F815-3-C2-IR	PLCVLEELFWGAPLFGQCSD	32.3	1.0	<0.1	35.6
F815-4-E10-IR	DLCGLEELFWGAALFGQCTS	35.4	1.0	<0.1	35.4
F815-3-A12-IR	QLCVLEKQLWGASLFWQCSCG	32.1	1.0	<0.1	35.3
F815-3-B8-IR	HLCVLEELFWGASLYGQCPG	33.6	1.0	<0.1	35.3
F815-3-B2-IR	HLCVLEELLWGASLFDQCSCG	31.0	1.0	<0.1	35.3
F815-3-C3-IR	HLCVLEELFWGVSLFGQCGG	30.1	1.0	<0.1	35.3
F815-3-A7-IR	HLCVLEELFWGASQWQGCSCG	33.1	1.0	<0.1	35.2
F815-4-F9-IR	RLCVLEEQFWGGALFGQCSCG	33.4	1.0	<0.1	35.2
F815-3-B7-IR	QLCVLEELFWGVSLFAQCSCG	32.0	1.0	<0.1	35.0
F815-4-E4-IR	HLCVLEELFWGAALFGQCFCG	28.0	1.0	<0.1	35.0
F815-4-E12-IR	YLCVLEELFWGASQFGQCSCG	28.0	0.9	<0.1	34.8
F815-4-F8-IR	HLCVLEELYWGASLFGQCSCG	33.8	1.0	<0.1	34.7
F815-3-C7-IR	HLCVLEERFWGVSLFGQCSCG	33.9	1.0	<0.1	34.7
F815-4-F10-IR	PLCVLEELFWGASRFGQCSCG	32.7	1.0	<0.1	34.7
F815-3-D11-IR	HLCVLEDLFWGASLFDQCSCG	35.4	1.1	<0.1	34.6
F815-4-E7-IR	HLCDLEVLFWGASLFGQCSCG	30.3	0.9	<0.1	34.6
F815-3-A10-IR	QLCILEEQFWGTSLFGYCSCG	34.0	1.1	<0.1	34.3
F815-3-B11-IR	ALCVLEELFWGESLFGQCSCG	33.7	1.1	<0.1	34.2

FIG. 2B-2

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR
	<u>HLCVLEELFWGASLFGYCSG</u>	--	--	--	--
F815-4-F11-IR	RLCVLEERFWGAALFGQCSG	31.8	1.0	33.7	<0.1
F815-3-A9-IR	PLCVLEELFWGASLFGQCSG	31.9	1.0	35.5	<0.1
F815-4-G11-IR	SLCVLEELFWGSRFGQCSG	32.3	1.0	34.4	<0.1
F815-3-D8-IR	HLCLEEQFWGASLFGYCFE	32.3	1.0	33.3	<0.1
F815-4-G4-IR	HLCVLEEQFWGASLFGQCSG	23.8	1.0	32.2	<0.1
F815-3-C8-IR	DLCLLELLWGASRFGQCSG	33.9	1.0	35.1	<0.1
F815-4-G12-IR	YLCVLEERFWGASLFGQCSG	31.7	1.0	33.5	<0.1
F815-3-D12-IR	HLCVLEEQFWGASLFGSCSG	33.3	1.0	34.8	<0.1
F815-4-F7-IR	QLCVLEEQFWGASLFGQCSG	33.3	1.0	34.3	<0.1
F815-4-F2-IR	HLCVLEELF*GESLFGYCSG	26.1	1.0	33.8	<0.1
F815-3-B9-IR	HLCVLEELFWGASLFGQCSG	33.6	1.1	35.7	<0.1
F815-4-H2-IR	PLCVLEELFWGASHFGQCSG	36.1	1.2	38.4	<0.1
F815-4-E11-IR	HLCVLEELVWGASLFGQCSG	33.2	1.1	35.4	<0.1
F815-4-G1-IR	QLCVLEELIWGASLFGQCSG	27.9	1.0	31.5	<0.1
F815-3-A11-IR	HLCGLEELFWGASLFGQCSG	37.7	1.2	40.1	<0.1
F815-4-F6-IR	HLCVLEELVWGESLFGQCSG	32.3	1.1	34.6	<0.1
F815-3-D9-IR	RLCVLEELYWGASLFGQCSG	31.4	1.0	32.5	<0.1
F815-3-C11-IR	RLCILEELFWGASLFGQCSG	33.4	1.1	35.7	<0.1
F815-4-G2-IR	HLCVLEELFWGATLFDQCSG	30.2	1.1	34.3	<0.1
F815-3-C9-IR	HLCFLEELFWGASLFGQCSG	29.7	1.0	31.4	<0.1
F815-4-H10-IR	HLCIVEELFWAAPLFGQCSG	31.9	0.9	27.6	<0.1
F815-4-F3-IR	HLCVLEELVWGASLFAQCSA	19.4	1.0	28.0	<0.1
F815-4-F5-IR	NLCALLELFWGASQFRYCPG	12.3	0.9	24.8	<0.1
F815-4-H1-IR	RLCVLEELFWGASLFGQCSG	6.9	1.0	15.8	0.1
F815-4-E5-IR	PLCVLEELFWGASLFGQCPG	3.5	1.0	13.6	0.1
F815-4-H5-IR	NLCVLEELFWGASLFGQCSG	5.5	1.0	13.1	0.1
F815-3-C10-IR	QLCVLG#RFWGGSLCGYCSG	3.5	1.1	5.2	0.2

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FIG. 2B-3

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Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGF9R	IR	IGFR/IR	IR/IGFR
		<u>HLCVLEELFWGASLFGYC</u> SG	39.1	1.8	27.7	0.1	15.4
	F815-4-F11-IGFR	PLCFLQELFGGASLGGYCSG	33.4	12.3	1.0	12.3	0.1
	F815-4-E12-IGFR	FMCGLQELVGGAAALGHCSG	33.7	15.1	1.7	8.9	0.1
	F815-4-H10-IGFR	PLCFLQELFGGSLSGYC	30.1	8.5	1.0	8.5	0.1
	F815-4-B7-IGFR	FLCGLEELAWGVSRSYCFG	35.2	23.9	4.8	5.0	0.2
	F815-3-B5-IGFR	PLCFLAELFSGSALGGDCSR	33.9	4.8	1.0	4.8	0.2
	F815-4-D12-IGFR	PLCVLQELFGGSLGGYCSG	33.6	7.0	1.8	3.9	0.3
	F815-4-C11-IGFR	QLCVLE#LFWGACLFGYCAG	13.9	4.6	1.8	2.6	0.4
	F815-4-C7-IGFR	FLCGLQELSGVASLFGQCSG	16.8	2.0	1.0	2.0	0.5
	F815-4-E7-IGFR	RVCVLEQLVWGASLFGA*SG	26.9	3.8	1.9	2.0	0.5
	F815-4-G7-IGFR	FYCGLEELSWGAAALFGYC	30.4	9.0	5.0	1.8	0.6
	F815-4-A10-IGFR	FLCGLEELSQGAVLFGHCY	30.8	3.7	2.2	1.7	0.6
	F815-3-B3-IGFR	HLCVLVGLFWDASLFGQCSG	7.6	1.0	2.0	0.5	2.0
	F815-3-G1-IGFR	QRCIRAAALFWCATLLGGCAG	20.5	1.0	2.0	0.5	2.0
	F815-4-G12-IGFR	HQIPDGMSQGAALRGNCSD	7.6	1.0	2.5	0.4	2.5
	F815-3-H1-IGFR	HLCVLEDELWGVSLFGYC	18.4	1.0	6.8	0.1	6.8

FIG. 2C

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Clone Parental/Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
F820-4-B5-IR	HLCVLEELFWGASLFGYCSCG	39.1	1.8	27.7	0.1 15.4
F820-4-A2-IR	HLCMLEEQFWGASLFSRCSG	28.1	0.9	17.9	<0.1 21.1
F820-4-E2-IR	TCAFWKNGSGVRRCSVTAVV	34.0	1.6	22.7	0.1 13.9
F820-4-D10-IR	PLCGLKN.SGVRLCSSPALV	21.3	0.7	9.0	0.1 13.4
F820-4-H7-IR	PLCLQEELFWGASLFGYCSCG	34.1	1.0	12.1	0.1 12.1
F820-4-G6-IR	PLCDLEELFWGASLFGDCPG	14.2	0.6	6.5	0.1 11.6
F820-4-C2-IR	DLCVLEELFWDGSLFASCSG	14.0	0.5	6.1	0.1 11.5
F820-4-B4-IR	PLCVLEEQWGTALFGSCTG	38.1	1.2	11.8	0.1 9.9
F820-4-C7-IR	PLCLVEELLWGASLFSQCTG	15.1	0.7	6.4	0.1 8.7
F820-4-F10-IR	PLCDLEELYWGAALFGSCSG	46.3	2.7	22.2	0.1 8.2
F820-4-G5-IR	GLCFLEEQFWGTSLFRDCPG	14.5	0.6	4.7	0.1 8.0
F820-4-F2-IR	PLCVVEELFWGASLYGQCSCG	8.8	0.6	4.4	0.1 7.5
F820-4-H8-IR	RLCVLEELFWGASRFRGCSG	11.7	0.6	4.2	0.1 7.4
F820-4-D7-IR	PLCVLEELHWGAALFGYCSCG	16.0	0.6	4.7	0.1 7.3
F820-4-B2-IR	NLCVVEELFWGASLFPNCSCG	14.5	0.8	5.9	0.1 7.1
F820-4-C3-IR	QLCVLEELFWGASMFEDCSG	5.0	0.4	2.4	0.2 6.9
F820-4-H4-IR	HLCVLEEQFWGASLFGQCSCG	37.5	1.1	7.5	0.2 6.6
F820-4-B10-IR	PLCVLEEIYWGAALFGDCYG	21.2	1.1	6.4	0.2 5.9
F820-4-A5-IR	PLCVLEELFWGLSLDKNCS	7.5	0.7	3.7	0.2 5.6
F820-4-F6-IR	QLCVLEELFWGASLFSGCSG	5.3	0.8	4.4	0.2 5.2
F820-4-F1-IR	PLCDLEALFWGESLFGGCSG	5.7	0.6	3.0	0.2 4.9
F820-4-A3-IR	HLCVLEEMFWGTSHFDGCSG	9.1	1.0	4.7	0.2 4.7
F820-4-D1-IR	DLCVLEELFWGAPLFGLCSCG	5.9	0.8	3.5	0.2 4.5
F820-4-F5-IR	DLCVLEELFWGVALYGGCSG	25.7	2.3	10.5	0.2 4.5
F820-4-F12-IR	QLCVLEELYWGASLFGHCSCG	3.7	0.6	2.7	0.2 4.2
F820-4-A11-IR	HLCVLEDRFWGASLFGPCSCG	11.3	0.6	2.2	0.3 3.5
F820-4-E8-IR	HLCGMEEMFWGVALLFRNCSCG	7.6	0.8	2.7	0.3 3.5
F820-4-H3-IR	PLCVLEQLYWGESLFFVYCSCG	8.0	1.2	4.3	0.3 3.5
	HLCVLEELFWGEALWGYCSCG	17.5	2.6	9.0	0.3 3.4

FIG. 2D-1

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Clone Parental/Design	Sequence	Ratios over Background		Comparisons	
		E-Tag --	IGF ₃ R --	IR --	IGFR/IR --
F820-4-A8-IR	HLCVLEELFWGASLFGYCSCG	6.4	0.7	2.4	0.3
F820-4-G1-IR	QLCVMEELFWGASRFGQCSCG	3.9	0.6	1.9	0.3
F820-4-F3-IR	HLCVLEELFWGASMFQCSCG	9.8	1.3	3.6	0.4
F820-4-D6-IR	QLCVLEEMFWGSRFVQCSA	5.4	1.2	3.2	0.4
F820-4-A1-IR	PLCILEELFWGEALFDQCGA	25.5	2.4	6.1	0.4
F820-4-H2-IR	YLCVQEELFWGASLFGYCSCV	15.9	1.6	4.1	0.4
F820-4-F4-IR	HLCVLEERFWGASLFGQCSCG	6.8	1.9	4.7	0.4
F820-4-B6-IR	QLCDLEELFWGASLFGYCPCG	4.1	0.8	1.9	0.4
F820-4-B11-IR	HLCVLEERFWGASLFGQCSCG	22.2	3.1	7.0	0.4
F820-4-H6-IR	QLCVLEELFWGASLFGQCSCG	4.1	1.1	2.4	0.5
F820-4-H9-IR	PLCVLEELFWGAAQFGQCSCG	3.1	0.9	1.9	0.5
F820-4-D3-IR	QLCDLEERFWGVSIFGLCSG	4.6	1.3	2.5	0.5
F820-4-C1-IR	QLCVLEEVFWGASLFGLCCTG	13.0	1.1	2.1	0.5
F820-4-D12-IR	QL.DLNTWSGLCLCSVTVRV	10.4	1.2	2.0	0.6
F820-4-B8-IR	DLCVLEESLWGKALFGYCSD	7.2	2.2	3.4	0.6
F820-4-C6-IR	HLCVLEEVFWGSSMFGDCSCG	13.9	2.5	2.8	0.9
F820-4-C10-IR	HLCDLEELFWGASLFGDCQG	5.3	2.6	2.9	0.9
F820-4-D4-IR	QLCVLDALMWGGCRLGHQCG	3.5	2.3	2.1	1.1
F820-4-E1-IR	QLCVLEEKFWGTSIFGDCMG	1.6	1.6	1.5	1.1
F820-4-B3-IR	HLCVLEEVFWGAAQFGSCSG	15.9	0.6	5.0	1.2
F820-4-D2-IR	QLCVLEELFWGSPMFGYCSCG	7.8	3.2	2.5	1.3
F820-4-C5-IR	HLCDLEELFWGASGFAQCYG	21.5	4.0	2.3	1.8

FIG. 2D-2

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Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR	IR/IGFR
A6L-3-C4-IR	HLCVLEELFWGASLFGYCSCG	36.9	1.0	40.5	<0.1	--	42.5
A6L-3-D7-IR	DLCVLEERFWGASLFGQCSCG	38.6	1.0	40.1	<0.1	--	40.7
A6L-3-A1-IR	QLCVLEELHWGASLFGYCSCG	39.6	1.1	44.8	<0.1	--	40.6
A6L-3-C1-IR	PLCVLEEQFWGASLFGQCSCG	37.3	1.0	40.3	<0.1	--	40.3
A6L-3-D5-IR	YLCDLEERFWGASLFGQCSCS	42.9	1.1	44.4	<0.1	--	40.2
A6L-3-A4-IR	HLCVLEELFWGASQFGQCSCG	26.7	1.1	42.2	<0.1	--	40.2
A6L-3-D3-IR	HLCVLEERFWGASLFGQCSCG	34.6	0.9	36.9	<0.1	--	39.8
A6L-3-B1-IR	HLCVMEELFWGTSLFGQCTG	33.9	1.0	38.7	<0.1	--	39.3
A6L-3-B5-IR	HLCVLEERFWGASLFGQCSCG	35.3	1.1	42.4	<0.1	--	38.6
A6L-3-B2-IR	HLCVLEERFWGASLFSQCSCG	38.1	1.1	42.7	<0.1	--	37.7
B6H-4-G12-IR	HLCVLEELFWGASLFGQCSCG	31.6	1.1	39.6	<0.1	--	36.7
B6C-4-H10-IR	QLCLLEELFWGAASFGQCSCG	38.5	1.1	41.1	<0.1	--	36.5
B6H-4-G8-IR	HLCVLEEMFWGASLFGQCSCG	31.7	1.1	39.7	<0.1	--	36.2
A6L-3-D6-IR	HLCVLEELFWGASLFSQCSCR	35.5	1.0	37.2	<0.1	--	36.1
B6C-4-F1-IR	QLCVLEELFWGASQFGYCSCG	32.9	1.1	38.7	<0.1	--	35.8
B6C-4-H3-IR	QLCALEEQFWGASLFSQCSCG	37.4	1.2	40.5	<0.1	--	34.8
B6H-4-E8-IR	QLCVLEELFWGASLFGYCSCG	30.2	1.0	35.7	<0.1	--	34.3
B6C-4-G1-IR	HLCVLEWFGDSLFGQCSCR	34.9	1.2	40.2	<0.1	--	33.7
B6H-4-E9-IR	HLCVLEERFWGASLFGQCSCG	34.4	1.2	38.8	<0.1	--	33.2
B6C-4-F5-IR	QLCELEEVFWGASLFDYCSCG	34.7	1.2	39.6	<0.1	--	32.8
B6C-4-F11-IR	HLCVLEELFWGASRFGQCSCG	34.0	1.2	37.2	<0.1	--	31.7
B6C-4-E6-IR	HLCVLEELFWGASLFGQCSCA	32.3	1.2	37.4	<0.1	--	30.6
B6C-4-E12-IR	HLCVLEELINGASRFGQCSCG	30.9	1.1	33.3	<0.1	--	30.2
B6C-4-G10-IR	HLCVLEELFWGGSFLFIQCSCG	33.0	1.3	40.3	<0.1	--	30.1
B6C-4-F8-IR	QLCVLEEQFWGASLFGNCSCG	36.4	1.4	39.8	<0.1	--	29.3
20C-3-B5-IR	HLCVLEERFWGAALFGQCSCG	26.6	1.1	32.5	<0.1	--	29.2
B6C-4-G3-IR	HLCILEEMFWGASLFGQCGG	34.0	1.4	38.8	<0.1	--	28.3
20C-3-B7-IR	PLCVLEELVWGASLFFVQCSCG	29.5	1.2	32.9	<0.1	--	28.3

FIG. 2E-1

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Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IR	IGFsR/IR/IGFR
20C-3-B4-IR	HLCVLEELFWGASLFGYCSCG	--	--	--	--
20C-3-C11-IR	NLCVLEELFWGESLFGQCSG	28.9	1.1	31.1	<0.1
B6C-4-G2-IR	HLCVLEEQFWGSLFGYCSC	30.2	1.1	31.0	<0.1
20C-3-B8-IR	HLCFLEEVFWGAALFAQCSG	29.4	1.3	35.3	<0.1
20C-3-C10-IR	HLCDLEVLFWGSALFGQCSG	28.5	1.1	31.2	<0.1
20C-3-B6-IR	HLCVMEELFWGASLFGQCSG	32.1	1.2	33.6	<0.1
A6L-3-A3-IR	HLCVLEERFWGASLFWQCSG	29.7	1.2	31.9	<0.1
A6L-3-B3-IR	HLCVLEEQYWGSLFGYCSC	14.4	1.1	28.3	<0.1
20C-3-A5-IR	PLCVLEEQFWGASLFGYCSC	38.7	1.7	43.4	<0.1
20C-3-B11-IR	QLCVLEELFWGESLFAQCLG	22.9	1.1	27.6	<0.1
20C-3-B3-IR	HLCVLEELFWGQSLFGHCSD	30.0	1.3	32.7	<0.1
20C-3-C12-IR	HLCVLEELVWGASLFGFCSC	29.3	1.2	31.2	<0.1
20C-3-C3-IR	LLCVLEEQFWGASLFGQCSG	29.6	1.3	31.8	<0.1
20C-3-C2-IR	RLCVLEELFWGESLFGQCSG	30.1	1.2	30.1	<0.1
20C-3-A11-IR	HLCVLEEMFWGASLFGNCSC	29.9	1.3	29.8	<0.1
20C-3-A4-IR	ELCFLEELFWGASLFGQCSG	25.9	1.2	27.4	<0.1
20C-3-A6-IR	HLCVLEELFWGASLYGQCS	27.2	1.2	27.5	<0.1
B6C-4-E4-IR	HLCVLEELFWGASLFAQCPG	26.1	1.2	27.5	<0.1
20C-3-A9-IR	NLCVLEELFWGASEFGQCSG	34.5	1.7	39.1	<0.1
B6C-3-C5-IR	DLCVLEELQWNGASLFRYCSC	29.7	1.3	29.3	<0.1
20C-3-B1-IR	HLCVLEEQFWGVALLFGNCSC	33.5	1.7	37.7	<0.1
20C-3-A10-IR	HLCVLEVQIWGASLFGQCSG	30.2	1.2	26.7	<0.1
20C-4-F1-IR	HLCVLEERFWGGALLFGQCTA	29.0	1.3	28.5	<0.1
20C-4-E1-IR	HLCVLEELFWGASLFGQCSG	29.1	1.4	29.5	<0.1
20C-3-B12-IR	QLCVLEELFWGTSLFGCSG	28.3	1.4	29.7	<0.1
20C-3-A8-IR	QLCVLEELFWGASLFGYCSC	27.0	1.3	25.8	<0.1
20C-3-A7-IR	HLCVLEELFWGASLFGQCS	21.1	1.1	21.2	0.1
B6C-4-E10-IR	FLCVLEELYWGASQFGQCSG	21.9	1.3	23.0	0.1
	HLCVLEEQFWGASLFGYCSC	35.2	2.2	38.0	0.1

FIG. 2E-2

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGF β R	IR	IGFR/IR	IR/IGFR
20C-3-A1-IR	<u>HLCVLEELFWGASLFGXC</u> SG	--	--	--	--	--
20C-3-C1-IR	RLCALEELFWGASLFGQCSG	21.0	1.1	17.6	0.1	16.6
A6L-3-D2-IR	HLCVLEELFWGAALFHQCSG	30.6	1.4	21.9	0.1	16.1
B6C-4-G12-IR	RLCVLEEQFWGASLFGQCSG	7.0	1.1	14.9	0.1	14.1
B6H-4-F9-IR	<u>QLCVLEELFWGSSRLGYC</u> SG	31.1	2.5	33.5	0.1	13.6
B6C-4-E3-IR	DLCVLEELFWGASLFGQCSG	39.3	3.6	43.1	0.1	12.1
20C-3-B10-IR	<u>QLCLLEEQFWGGS</u> LFGQCSG	34.6	5.3	40.0	0.1	7.6
20C-3-A3-IR	HLCVLEELFWGTSLFGQCSG	29.9	16.9	31.7	0.5	1.9
	RLCVLEELVWGASLFDQCSR	28.4	19.1	25.3	0.8	1.3

FIG. 2E-3

Clone Design	Sequence	Ratios over Background			Comparisons		
		E-Tag --	IGF β R --	IR --	IGFR/IR --	IR/IGFR --	
R20 α -3-20A4-IR	EIEAEWGRVRCCLVYGRCVGG	50.2	1.6	23.1	0.1	14.4	45/163
R20 β -4-A7-IR	EIEAEWGRVRCCLVYGRCVGG	44.2	1.3	24.0	0.1	18.5	
R20 β -4-D8-IR	WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	0.1	17.3	

FIG. 3A

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Clone Parental/Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGF ₅ R	IR	IGF ₅ R/IR	IR/IGF ₅ R	IR/IGF ₅ R
D815-4-A8-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	44.8	1.4	24.2	<0.1	17.3	
D815-4-D10-IR	<u>WLDLEWAQVQCEVYGRGCP</u> S	48.0	1.0	48.4	<0.1	48.4	
D815-4-D9-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	49.2	1.0	48.2	<0.1	48.2	
D815-4-A11-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	47.5	1.0	48.0	<0.1	48.0	
D815-4-A11-IR	<u>RLDEEWAWVQCEVYGRGCP</u> S	47.9	1.0	48.0	<0.1	48.0	
D815-4-E12-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	49.0	1.0	47.6	<0.1	47.6	
D815-4-B7-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	45.4	1.0	47.2	<0.1	47.2	
D815-4-D11-IR	<u>WLDQEWAWVQCEVYGRGCP</u> A	49.5	1.0	47.0	<0.1	47.0	
D815-4-D12-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	48.1	1.0	46.6	<0.1	46.6	
D815-4-F8-IR	<u>WLDQEWAWVQCEVYGRGCP</u> A	47.8	1.0	46.4	<0.1	46.4	
D815-4-A9-IR	<u>SLDQEWAWVQCEVYGRGCP</u> S	47.7	1.0	45.8	<0.1	45.8	
D815-4-E9-IR	<u>WLDQEWAWVQCEVYGRGCP</u> P	47.8	1.0	45.8	<0.1	45.8	
D815-4-B10-IR	<u>WLDQEWAWVQCEVYGRGCP</u> Y	49.0	1.0	45.6	<0.1	45.6	
D815-4-H8-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	49.0	1.0	45.6	<0.1	45.6	
D815-4-E10-IR	<u>SLDQEWAWVQCEVYGRGCP</u> S	47.0	1.0	45.6	<0.1	45.6	
D815-4-D7-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	44.5	1.0	45.4	<0.1	45.4	
D815-4-G9-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	44.2	1.0	44.2	<0.1	44.2	
D815-4-G12-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	44.3	1.0	43.7	<0.1	43.7	
D815-4-E11-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	45.5	1.0	43.0	<0.1	43.0	
D815-4-H7-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	46.2	1.0	43.0	<0.1	43.0	
D815-4-F12-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	47.2	1.0	42.6	<0.1	42.6	
D815-4-E8-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	47.9	1.0	42.6	<0.1	42.6	
D815-4-F9-IR	<u>QLDQEWAWVQCEVYGRGCP</u> S	46.4	1.0	41.8	<0.1	41.8	
D815-4-A10-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	47.3	1.0	41.2	<0.1	41.2	
D815-4-C7-IR	<u>QLEQEWAWVQCEVYGRGCP</u> S	37.7	1.0	40.0	<0.1	40.0	
D815-4-H10-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	47.0	1.0	39.8	<0.1	39.8	
D815-4-C9-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	44.2	1.0	39.8	<0.1	39.8	
D815-4-F11-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	40.4	1.0	39.2	<0.1	39.2	
D815-4-H12-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	45.4	1.0	38.6	<0.1	38.6	
D815-4-A7-IR	<u>SLDQEWAWVQCEVYGRGCP</u> S	37.3	1.0	37.3	<0.1	37.3	
D815-4-H11-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	2.4	1.0	37.2	<0.1	37.2	
D815-4-F7-IR	<u>WLDQEWAWVQCEVYGRGCP</u> S	32.4	1.0	34.7	<0.1	34.7	

FIG. 3B-1

Clone	Parental/Design	Sequence	Ratios over Background		Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
D815-4-G8-IR		WLDQEWAWVQCEVYGRGCPS	--	--	--	--
D815-4-G7-IR		QLDQEWARVRCEVWGRGCSS	27.8	1.0	33.6	<0.1 33.6
D815-4-G11-IR		WLDLEWAQVQCKVYGRGCPS	34.7	1.0	32.3	<0.1 32.3
D815-4-E7-IR		WLDEEWAWVQCQVYGRGCPS	30.7	1.0	28.6	<0.1 28.6
D815-4-A12-IR		WLDQEWAWVQCEVWGRGCAF	33.0	1.0	26.4	<0.1 26.4
D815-4-B11-IR		WLDREWAQVQCEVYGRGCLS	28.4	1.0	19.0	0.1 19.0
D815-4-D8-IR		WLDAEWVQCEVYGRGCRP	22.1	1.0	18.8	0.1 18.8
		SLDREWAYVQCQVYGRGCSS	20.8	1.0	14.6	0.1 14.6

FIG. 3B-2

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Clone Parental/Design	Sequence	Ratios over Background			Comparisons		
		E-Tag	IGFsR	IR	IGF ¹ R	IR/IGF ¹ R	IR/IGF ¹ R
D820-3-H2-IR	WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	0.1	17.2	
D820-3-C4-IR	RLDLEWANIQCEVYGRGCPS	23.9	1.0	40.0	<0.1	40.0	
D820-3-C3-IR	WLEQEWARVQCEVYGRGCSS	31.0	1.0	39.5	<0.1	39.5	
D820-3-G6-IR	WLEQEWILVECEVYGRGCPT	35.2	1.0	39.4	<0.1	39.4	
D820-3-D2-IR	WLEQEWAVQCEVWGRGCPS	33.8	1.0	38.8	<0.1	38.8	
D820-3-D3-IR	WLDQEWEWIQCEVYGRGCPL	35.6	1.0	37.8	<0.1	37.8	
D820-3-B5-IR	LJDEEWAQIECEIYGRGCPS	34.8	1.0	37.7	<0.1	37.7	
D820-3-E2-IR	ALEEAWAVQCEVYGRGCHF	34.1	1.0	37.1	<0.1	37.1	
D820-3-B3-IR	C?EQEWGLVQCEVYGRGCPS	34.4	1.0	37.0	<0.1	37.0	
D820-3-B6-IR	WLEQEWAVQCEVYGRGCPS	33.6	1.0	36.7	<0.1	36.7	
D820-3-D4-IR	WLEHEWAQVQCEVWGRGCPY	31.2	1.0	36.6	<0.1	36.6	
D820-3-C2-IR	WLEQEWAEVRCEVYGRGCPR	32.0	1.0	36.2	<0.1	36.2	
D820-3-F6-IR	?LEQEWAVQCEVYGRGCPS	33.7	1.0	35.6	<0.1	35.6	
D820-3-D5-IR	WLEQEWAGIQCKVYGRGCPS	30.8	1.0	35.2	<0.1	35.2	
D820-3-F5-IR	RLEQEWAVQCEVWGRGCLP	30.5	1.0	34.8	<0.1	34.8	
D820-3-H3-IR	QLDHEWAGIQCEVWGRGCPS	29.8	1.0	34.6	<0.1	34.6	
D820-3-G2-IR	WLEQEWAGIQCEVYGAGCRS	30.2	1.0	33.8	<0.1	33.8	
D820-3-H6-IR	SLEQEWAVQCVVYGRGCPI	31.3	1.0	33.0	<0.1	33.0	
D820-3-F3-IR	WLEQEWQVLCVYGRGCPY	30.3	1.0	32.2	<0.1	32.2	
D820-3-B4-IR	WLEQEWAVQCEVYGRGCA?	28.6	1.0	30.7	<0.1	30.7	
D820-3-C5-IR	WMDQEWAVQCEVYGRGCPS	33.1	1.0	30.5	<0.1	30.5	
D820-3-F4-IR	QLDQEWAWIQCEVYGRNCRT	29.1	1.0	30.3	<0.1	30.3	
D820-3-H5-IR	TLEQEWAVQCEVYGRGCLS	25.9	1.0	29.5	<0.1	29.5	
D820-3-A6-IR	RLEQEWAVQCEVWGRGCLS	26.3	1.0	28.6	<0.1	28.6	
D820-3-A2-IR	WLDQEWALVQCEVYGRGCPA	24.8	1.0	26.0	<0.1	26.0	
D820-3-G5-IR	WLDQEWAVQCEVYGRGCPA	23.7	1.0	25.6	<0.1	25.6	
D820-3-G3-IR	WLEQEWAVQCEVYGRGCPS	22.6	1.0	25.0	<0.1	25.0	
D820-3-E3-IR	RLEEAWAVQCVYGRGCPS	22.2	1.0	23.9	<0.1	23.9	
	WLEQEWVRIQCEVYGRGCPS	20.6	1.0	22.7	<0.1	22.7	

FIG. 3C-1

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Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
D820-3-E5-IR		<u>WLDQEWAWVQCEVYGRGCPS</u>	44.8	1.4	24.2	0.1	17.2
D820-3-D1-IR		<u>WLEQEWTVVQCEVYGCPCPS</u>	25.9	1.0	22.6	<0.1	22.6
D820-3-E1-IR		<u>WLEKEWAGVQCEIYGRGCPS</u>	27.3	1.0	22.4	<0.1	22.4
D820-3-F1-IR		<u>WLEEEWAWVRCEVYGRGCQS</u>	22.4	1.0	21.9	<0.1	21.9
D820-3-B2-IR		<u>WLEHEWAIQCELYGRGCTY</u>	22.0	1.0	21.0	<0.1	21.0
D820-3-A3-IR		<u>WLEQEWAWVQCEVYGRGCPS</u>	13.1	1.0	18.4	0.1	18.4
D820-3-H4-IR		<u>WLEQEWAIQCEIYGRGCQS</u>	23.5	1.0	18.4	0.1	18.4
D820-3-G1-IR		<u>WLDDEWAIQCEIYGRGCQS</u>	25.6	1.0	17.5	0.1	17.5
D820-3-C1-IR		<u>QLEEEWAGVQCEVYGRECP</u>	14.5	1.0	16.3	0.1	16.3
D820-3-A1-IR		<u>WLEQEWLLVQCGVYGRGCPS</u>	27.8	1.0	13.9	0.1	13.9
D820-3-A5-IR		<u>WLDQEWAWIQCEVYGRGCRS</u>	14.7	1.0	12.8	0.1	12.8
D820-3-H1-IR		<u>WLEQEWAVQCEVSGRGCP</u>	6.4	1.0	6.3	0.2	6.3
D820-3-A4-IR		<u>W?DQEWALIQCEVYGRGCPS</u>	13.7	1.0	6.2	0.2	6.2
D820-4-E12-IR		<u>SLDEEWAGVLCVYGRGCPF</u>	6.0	1.0	4.3	0.2	4.3
D820-4-B12-IR		<u>SVDQELEWLMCHFQGRVCPS</u>	34.9	9.0	10.9	0.8	1.2
		<u>WLEQERAWIWCETQSGCRA</u>	32.2	8.6	1.0	8.6	0.1

FIG. 3C-2

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Clone Parental/Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
D820-3-D5-IGFR	WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	0.1	17.3
D820-3-E4-IGFR	WVNQALGGVQSDVQGRRCQS	29.6	3.8	1.0	3.8	0.3
D820-3-C5-IGFR	LLDHEWPWVGCEVCGRGSLS	27.1	3.2	1.0	3.2	0.3
D820-3-F4-IGFR	WLHQELAWVRGEGYPRGRS	25.0	3.1	1.0	3.1	0.3
D820-3-F6-IGFR	WLGHDAWVQCEVYGLGPC	3.9	2.7	1.0	2.7	0.4
D820-3-G4-IGFR	WIDQEGVRVQCEA*GRAFPS	26.7	2.6	1.0	2.6	0.4
D820-3-E2-IGFR	WRDEEWAVVQGVVQGRGWP	3.8	2.6	1.0	2.6	0.4
D820-3-G6-IGFR	RLGVENSWFQKVGGRDSTS	15.3	2.6	1.0	2.6	0.4
D820-4-E11-IGFR	WLAQGWAGVQCVCVYGRGCRN	20.3	2.4	1.0	2.4	0.4
D820-4-H11-IGFR	WLEEE*AGIQCV?GRGCPS	12.6	1.0	3.0	0.3	3.0
D820-4-D11-IGFR	WLDQEWVQCEVWGRGCLS	8.1	1.0	4.6	0.2	4.6
D820-4-A8-IGFR	RLEQEWALIQCEVYGRGCPS	4.5	1.0	5.3	0.2	5.3
D820-4-F9-IGFR	WLEEEWAQVQCVCVYGRGCAS	3.2	1.0	5.5	0.2	5.5
D820-4-C8-IGFR	WLDLE*EWLQCEVYGRGCAT	9.4	1.0	5.8	0.2	5.8
D820-4-D9-IGFR	WLEQEWQVRCEVYGRGCPS	11.6	1.0	5.9	0.2	5.9
D820-4-D7-IGFR	WLEEEWAQVQCVCVYGRGCPS	10.1	1.0	8.9	0.1	8.9
D820-4-H9-IGFR	WLDQEWARVQCEVWGRGCTY	34.1	3.5	33.4	0.1	9.5
D820-4-E10-IGFR	YLD?EWAQVQCEVYGLGCQS	18.4	1.0	10.1	0.1	10.1
D820-4-E7-IGFR	WLDVE*AWVQCEVWGRGCPS	26.7	2.6	27.0	0.1	10.4
D820-4-H8-IGFR	WLEQEWER?QCEVYGRGCPP	31.9	3.0	32.2	0.1	10.7
D820-4-A11-IGFR	WLEEEWAQVQCVCVYGRGCLS	16.1	1.0	11.7	0.1	11.7
D820-4-C9-IGFR	WLDQEWAWIQCEVYGRGCPS	8.0	1.0	12.5	0.1	12.5
D820-4-E9-IGFR	?LEHEWAQIQCEV?GRGCQS	19.6	1.0	14.9	0.1	14.9
D820-4-B10-IGFR	WL?QEWAWIQCEVYGRGCPP	19.3	1.0	17.3	0.1	17.3
D820-4-F10-IGFR	WLD?EWAQVQCEVYGRGCPS	19.3	1.0	21.5	<0.1	21.5
D820-4-B9-IGFR	GLEQGCPCWVGLVQCRGCPS	27.8	1.0	25.7	<0.1	25.7
D820-4-G8-IGFR	WLEEEWAQVQCVCVYGHGCS	31.7	1.0	26.5	<0.1	26.5
	WLDQEWAWIQCEVYGRGCSS	25.6	1.0	29.3	<0.1	29.3

FIG. 3D-1

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
D820-4-G9-IGFR		WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	0.1	17.3
D820-4-C10-IGFR		WLDQEWAVQCEVWGRGCPS	36.8	1.0	29.6	<0.1	29.6
D820-4-A9-IGFR		WLDLEWVQCEVYGRGCPT	32.6	1.0	31.3	<0.1	31.3
D820-4-B8-IGFR		WLEQEWASVQCEVYGRGCPS	20.4	1.0	31.4	<0.1	31.4
D820-4-F8-IGFR		WLDLEWEQIKCKVYGRGCPF	31.1	1.0	32.7	<0.1	32.7
D820-4-H7-IGFR		WLEQEWAIQIQCIYGRGCPS	28.3	1.0	32.9	<0.1	32.9
D820-4-E8-IGFR		WLEQEWALVLCVYGHGCPA	34.1	1.0	32.9	<0.1	32.9
D820-4-G10-IGFR		WLEQEWAIQIQCEVWGRGCSS	26.6	1.0	33.2	<0.1	33.2
D820-4-D10-IGFR		WLE?EWEVQCEVYGRGC?S	37.5	1.0	33.2	<0.1	33.2
D820-4-D8-IGFR		WLEQEWAVQCDVYGRGCPS	36.6	1.0	33.5	<0.1	33.5
D820-4-A10-IGFR		WLEQE*ARVQCEVWGRGCPS	23.7	1.0	34.6	<0.1	34.6
D820-4-B7-IGFR		WL?QEWARVHCEVWGRP?QC	29.4	1.0	35.5	<0.1	35.5
D820-4-E12-IGFR		PLEHEWAWVQCVYGRGCRS	35.4	1.0	36.9	<0.1	36.9
D820-4-H10-IGFR		SLE?EAWVQCEV?GRGCP?	37.0	1.0	37.0	<0.1	37.0
D820-4-F12-IGFR		WLDQEWVRVQCEVWGRGCPS	36.8	1.0	37.1	<0.1	37.1
D820-4-F7-IGFR		SLDKEWAVKCEVYGRGCPS	36.9	1.0	37.3	<0.1	37.3
D820-4-G12-IGFR		LGDQEWAVWEV#GRGWPS	34.4	1.0	37.5	<0.1	37.5
D820-4-D12-IGFR		WLEEWAQIRCGVYGRGCPS	30.3	1.0	37.8	<0.1	37.8
D820-4-A12-IGFR		WLEEE*GWVQCEVWGRGCPP	37.2	1.0	38.6	<0.1	38.6
D820-4-C12-IGFR		CLDQEWAVQCPVYGRGCPS	30.4	1.0	39.3	<0.1	39.3
D820-4-A7-IGFR		QLELEWARVQCEVWDRGCPS	37.1	1.0	39.6	<0.1	39.6
D820-4-B12-IGFR		RLEQEWAWIQCEVYGRGCRF	35.4	1.0	40.8	<0.1	40.8
		SLEHE*ANVQCKVYGRGC?S	36.2	1.0	41.4	<0.1	41.4

FIG. 3D-2

Clone	Parental/Design	Sequence	Ratios over Background			Comparisons	
			E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
B6-4-G12-IR		WLDQEWAWVQCEVYGRGCPS	44.8	1.4	24.2	<0.1	17.3
B6-3-A11-IR		WLDQEWAWIQCEVYGRGCPP	4.4	1.0	6.9	0.1	7.1
		WLDQEWAVRCEVYGRGCPS	7.3	1.0	6.3	0.2	6.3

FIG. 3E

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF ₃ R	IGFR/IR	IR/IGFR
R20-4-C10-IGFR	XXXXXXXXXXXXXXXXXXXXXXXX PKGTRFRGDVDVDWDGYSWLA	--	--	--	--
		37.8	3.8	--	--

FIG. 4A-1

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGF ₃ R	IGFR/IR	IR/IGFR
20F-4-B7-IGFR	XXXXXXXXXXXXXXXXXXXXXXXX TPIPAGGINIASWGGYTWS	10.9	3.7	--	--
20F-4-E4-IGFR	HRGTVTGVVVARWPGYEWLS	8.9	4.7	7.3	0.1
20F-4-E12-IGFR	SDVWAQPQRNDWPGYHWLS	9.7	4.7	6.3	0.2
20F-4-F4-IGFR	HRGTVTGVVVARWPGYEWLS	13.9	10.1	6.0	0.2
20F-4-F7-IGFR	SDVWAQPQRNDWPGYHWLS	13.7	3.9	5.6	0.2
20F-4-E7-IGFR	RPHRINPQDDAVWPGYLWL	7.2	2.5	5.1	0.2
20F-4-F11-IGFR	HRGTVTGVVVARWPGYEWLS	17.6	16.2	4.7	0.2
20F-4-D10-IGFR	FGRGYGGDGGYWSGYEWLA	9.8	2.4	4.6	0.2
20F-4-B3-IGFR	DGLVVKSGREWPGYGLER.A	17.3	14.4	4.1	0.2
20F-4-B12-IGFR	DGSIV.VSSSVGWPGYEWLM	10.1	9.9	4.0	0.2
20F-3-A9-IGFR	WQQANLSNGGRWGGYDWLM	6.6	2.7	4.0	0.2
20F-4-G2-IGFR	FGRGYGGDGGYWSGYEWLA	5.1	1.3	4.0	0.2
20F-4-D11-IGFR	VNYEMDRVPPMPWGGYWWLS	5.0	1.0	2.7	0.4
20F-4-G4-IGFR	MGGGLWVGVIWPGYSWLSQ	3.9	0.9	2.3	0.4
20F-4-G12-IGFR	SDVWAQPQRNDWPGYHWLS	3.2	0.9	1.8	0.6
				1.5	0.7

FIG. 4A-2

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Clone
Design
R20β-4-A4-IR
R20β-4-F2-IR
R20β-4-E8-IR

Sequence
XXXXXXXXXXXXXXXXXXXXX
WPGYLFEEALQDWRGSTD
SMFVAGSDRWPGYGVLDWL
VRGFQGGTVWPGYEWLRNAA

Ratios over Background		Comparisons	
E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
--	--	--	--
11.9	17.5	1.4	12.5 0.1
16.4	13.9	3.1	4.5 0.2
41.0	34.9	3.6	9.7 0.1

FIG. 4B-1

Clone
Design
20F-4-H10-IR
20F-4-C10-IR

Sequence
XXXXXXXXXXXXXXXXXXXXX
LDLASGDSWLGYDVLRGWLS
IHSSDGIGAWGGYAWFRDVA

Ratios over Background		Comparisons	
E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
--	--	--	--
10.2	3.1	2.4	1.3 0.8
23.4	9.6	4.1	2.3 0.4

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FIG. 4B-2

Clone
Design
R20β-4-D10-IR
R20β-4-D9b-IR
R20β-4-H4-IR
R20β-4-A2-IR

Sequence
XXXXXXXXXXXXXXXXXXXXX
LGPLLRWGSEVCGVWPDICE
PFGFGRWWGIPRMWYRNS
WWGGRRNRWWLERWGLGGER
GRVALWGPVWPRWWFMSRPV

Ratios over Background		Comparisons	
E-Tag	IGFsR	IR	IGFR/IR IR/IGFR
--	--	--	--
21.5	1.0	8.0	0.1 8.0
32.6	6.8	15.1	0.5 2.2
11.6	1.7	3.6	0.5 2.1
17.1	2.6	5.2	0.5 2.0

FIG. 4C

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGF _s R	IR	IGFR/IR	IR/IGFR
R40-3-40A2-IR	XXXXXXXXXXXXXXXXXXXXXXXXXXXXX RGTRDRLWKSGGFAIVPRWPCFSYHCLVEWITKTGSPG	44.6	1.5	2.7	0.6	1.8
R40-4-40F10-IR	XXXXXXXXXXXXXXXXXXXXXXXXXXXXX GRTSMAFVPPRHLPQELAPRPVRNHAWLVGGG	46.4	1.9	2.1	0.9	1.1

FIG. 4D

Clone Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
	XXXXXXXXXXXXXXXXXXXX	--	--	--	--
R20-4-F10-IGFR	CLGAGSFRAGILCLGGLPVS	35.5	6.0	--	--
R20-4-F7-IGFR	GFWATACGGQICEELGLKP	29.1	4.7	--	--
R20-4-H9-IGFR	DLFCAYMAQALGLGQDLSCG	25.7	3.0	--	--
R20-3-A4-IGFR	RHLLLPOIWIAS*GGWGMG	15.6	2.7	--	--

FIG. 4E

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag --	IGF _s R --	IR --	IGFR/IR --	IR/IGFR --
20C-3-H3-IGFR	XXXXXXXXXXXXXXXXXXXXXXXXXX	28.5	26.6	1.0	26.6	<0.1
20C-3-F4-IGFR	DHRLCGTDEYLMQDLFVRGLCRLIW	34.4	27.5	1.2	23.1	<0.1
20C-4-C10-IGFR	GLLFCKQLFTLAGLQPEAGCVSSSR	35.5	24.4	1.3	19.2	0.1
20C-3-G5-IGFR	IWIACLDELLRGQVWSSCRRRAPIG	29.3	21.1	1.1	18.7	0.1
20C-3-A2-IGFR	DWLRCGLVILSGGLTELANTCVQG	33.9	18.3	1.1	16.9	0.1
20C-3-B4-IGFR	WFSFCLGGLLQAQEWVWGRDVGCI	39.8	29.1	1.9	15.2	0.1
20C-3-C6-IGFR	GYSWLRDVLMEKQAQLKREGSVGRQ	34.8	20.9	1.4	14.9	0.1
20C-3-E2-IGFR	FLTRLRLERLGLS*ERGEAGGPYAQA	34.8	28.1	2.0	14.2	0.1
20C-3-A3-IGFR	FSGFCMGLERLSQVSLGYCGAGQGG	33.7	14.3	1.2	12.4	0.1
20C-3-B1-IGFR	ISFRCLFVLAGMHPCPVDVGGEGF	30.2	9.8	0.9	11.2	0.1
20C-3-F5-IGFR	NTPNCSQDWQGESGFMAILLALTCK	35.5	31.9	3.9	8.2	0.1
20C-4-A7-IGFR	LQGFCELLATVTGTGLGCLDYQPI	33.3	19.3	2.8	6.9	0.1
20C-4-F8-IGFR	GSSICNLLARAQIVELALCEMGVQE	30.5	27.7	5.3	5.2	0.2
20C-4-G11-IGFR	LSFACLLSQLSGVWVLPDCLLGED	24.7	13.3	2.8	4.7	0.2
20C-3-E1-IGFR	GEHFCQLLMSLCGDDCGPVNCGGGS	34.0	5.1	1.6	3.1	0.3
20C-3-B6-IGFR	GWFECLLASLVLPQGRSRASAVC	37.3	32.8	13.7	2.4	0.4
	YRQECACSVGAVGFLCGLACLARSG					

FIG. 4F-1

Clone Design	Sequence	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	XXXXXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--
40F-4-D1-IGFR	LSCLAYSRHGIWRPSTDGLGRSVGECSVSTRWGYDNFE	4.9	4.6	0.3	13.1	0.1
40F-4-B1-IGFR	GLDHSDAVGVHLGFAWPA.ARGWEAGGLEDTWAGYDWL	4.1	3.0	0.2	13.1	0.1
40F-4-D10-IGFR	W.GYAWLS	4.9	4.5	0.4	11.7	0.1
40F-3-A3-IGFR	LSCLAYSRHGIWRPSTDGLGRSVGECSVSTRWGYDNFE	2.6	2.0	0.3	7.9	0.1
40F-4-C4-IGFR	EAMAVGLQCPARFVRAAAHGDGGSWGQDHV.AWGGYWWLG	3.8	2.0	0.5	4.1	0.2

FIG. 4F-2

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Clone	Parental/Design	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	<u>HLCVLEELFWGASLFGYC</u> SG	39.1	1.8	27.7	0.1	15.4
	HFYVLVERLSGASLFGSGA	34.6	7.9	1.0	7.9	0.1
	HRFVREGLLWGAYQFCYCSG	14.9	1.0	2.0	0.5	2.0
	FQSLLEELVWGAPLFRYGTG	35.2	1.0	2.0	0.5	2.0
	HLSVLEELSWGASLFGQWAG	5.4	1.0	2.1	0.5	2.1

FIG. 4G

Clone	Parental/Design	Sequence
		<u>HLCVLEELFWGASLFGYC</u> SG
F815-4-G11-IGFR		HFYVLVERLSGASLFGSGA
F815-3-D1-IGFR		HRFVREGLLWGAYQFCYCSG
F815-4-C12-IGFR		FQSLLEELVWGAPLFRYGTG
F815-4-A11-IGFR		HLSVLEELSWGASLFGQWAG

Clone	Parental/Design	Ratios over Background			Comparisons	
		E-Tag	IGFsR	IR	IGFR/IR	IR/IGFR
	<u>HLSVLEELSWGASLFGQ</u> WAG	5.4	1.0	2.1	0.5	2.1
	NLCRLEELAWGASLFGQCAG	16.3	1.0	2.7	0.4	2.6
	APVSTEELRWGALLFGQWAG	15.6	1.0	2.6	0.4	2.5
	HLSVLEERWWRESLFGQWAG	13.6	2.8	6.7	0.4	2.3
	HLSVLEERWWRAALFGQWAG	13.9	4.8	9.5	0.5	2.0
	HLSILEEQWRESLFGQWAG	16.9	1.3	2.3	0.6	1.8
	HMSVEELSWASLFGKQAG	11.3	1.3	2.3	0.6	1.7
	HLSLEERWWRATLFGQWAG	13.2	1.3	2.1	0.6	1.7
	HLSVLEELWWRESLFGQWAG	15.4	2.0	3.2	0.6	1.6
	HLSLLEEQWRESLFGQWAG	14.6	4.6	6.9	0.7	1.5
	HLSVLEERWWRETlFGQWAG	14.0	3.1	3.9	0.8	1.3
	HLSVLEEQWRESLFGQWAG	14.3	2.3	2.9	0.8	1.3
	HLSVLEEQWW.ESLFGQWAG	12.0	1.4	1.7	0.8	1.2
	HLSVLEELWWREALFGQWAG	13.6	1.2	1.5	0.8	1.2
	HLSVLEERWWRATLFGENAG	14.5	1.4	1.6	0.9	1.1
	HL.VLEELLWGVSLFRQWAG	8.4	1.4	1.5	1.0	1.1
	HLSALEEQWWRATLFGQWAG	14.1	2.8	2.9	1.0	1.0
	HLSVLEERWWRATLLESQ	14.7	1.4	1.4	1.0	1.0
	HLSALEELWWRETlFGQWAG	14.1	7.5	7.0	1.1	0.9
	HLSVLEELWWRESLFGKWAG	13.6	11.0	8.6	1.3	0.8
	HLSVLEEAWWRESLFGHWAG	15.5	7.9	6.0	1.3	0.8
	HMSQEELWWRATLFGQWAG	18.2	3.8	2.7	1.4	0.7
	HLSVLEERWWRETlFGENAG	16.5	12.9	8.2	1.7	0.6
	HRSVLKQLSWGASLFGQWAG	11.5	5.3	0.7	7.4	0.1

FIG. 4H

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Clone Parental/Design	Sequence	Ratios over Background		Comparisons	
		E-Tag	IGFsR	IGFR/IR	IR/IGFR
NNKH-2-C5-IGFR	HLSVLEELSWGASLFGQWAG	5.4	1.0	2.1	2.1
NNKH-2-D9-IGFR	HL*VLEELSWGASLVGQWAV	7.3	0.9	0.7	1.3
NNKH-2-H12-IGFR	HLSVLEEL*LGASMFGLWAG	4.1	0.5	0.4	1.3
NNKH-2-D10-IGFR	HLSVLKELSW*ASLFGQWAG	5.0	1.3	1.1	1.2
NNKH-2-G9-IGFR	HLSALEELSWGASLFGQWAG	4.8	2.1	1.9	1.1
NNKH-2-C6-IGFR	HLSVLAELS*GALLFGQWAG	1.9	1.4	1.3	1.1
NNKH-2-C7-IGFR	RLSVLEQLSWGASLFGPWAG	18.2	1.0	0.9	1.1
NNKH-2-F11-IGFR	HL*VLVQPSWGASLFGQWAG	21.8	1.3	1.3	1.0
NNKH-2-H3-IGFR	HQSVLEELSR*ASLFGQWAG	6.7	1.3	1.4	0.9
NNKH-2-B8-IGFR	DMSVLGGLSWGA*LFGQWSG	4.7	0.7	0.8	0.9
NNKH-2-B12-IGFR	HLSVREGQLWRASMFGRWAG	17.5	3.7	5.2	0.7
NNKH-2-F9-IGFR	QLSVLVEL*WGASLFGPWAA	1.2	1.0	2.9	0.3
	HLSVGEELSW*VALLGQWAR	3.7	0.6	2.1	0.3

FIG. 4I

D Name	Clonal Name	Formula #	K ₄ (μ M) HIR	PO ₄	Fat Cell Assay	Activity	K ₄ (μ M) HIGFR	Ratio IGF/IR	Sequence
D101	20D3	1	0.51 0.27				13 11	25 41	KIGGGQHGDGNFYDWFVEALAKK (ϵ -biotin)
D102	20D1	1	1.2 0.97				7.4 16	6.2 16	KVLQARHGCDSDCFYEWFAKK (ϵ -biotin)
D103	B8	1	0.74				15	20	KWSALLSMDTGYAWFDDAVKK (ϵ -biotin)
D104	E7	1	20				>20	>1	KGHSWALVRHVDRLFYEWFDLKK (ϵ -biotin)
D105	H8	1	2.8				12	4.3	KRDKPTDQEEQNWSFYEFWRHKK (ϵ -biotin)
D106	20F1	1	0.97				6.2	6.4	KVFWNCRSQQLDFYEWFFQAOKK (ϵ -biotin)
D107	40G11	1	1.1	YES		Antagonist	9.7	8.8	KLESHYVPPQAALDRLFYSWFSKK (ϵ -biotin)
D108	3G11	1	2.3			Antagonist	19	8.3	KFYGWSRQLSLTPRDDWGLPKK (ϵ -biotin)
D109	20H1	1	3.6			Antagonist	12	3.3	KSAPGLVSNKQDGLFYSWFREKK (ϵ -biotin)
D110	G3	1	0.84			Antagonist	1.4	1.7	KRGGIFYEFESALRKHGAGKK (ϵ -biotin)
D111	D2	1	0.62				3.2	5.2	KDPERMQSDVGFEWFRANGKK (ϵ -biotin)
D112	IGFR C1 A65-4-C1	1	0.49 0.19			Neutral	0.05* 0.02*	0.1 0.1	DYKDCWARPCGDAAFNFDWFVQQASKK (ϵ -biotin)
D113	IGFR H2 A65-4-1+2	1	0.75		-20 μ M	Agonist	5.4	7.2	DYKDVFTSAVFENFYDWFVRQVSKK (ϵ -biotin)
D114	IGFR A6	1	8.1			Neutral	>20	>2.5	SAKNFYDWFYKK (ϵ -biotin)
D115	IGFR D5	1	8.1				>20	>2.5	ADKNFYDWFMAKK (ϵ -biotin)
D116	IGFR JBA5	9	4.4 cycli		>20 μ M	Agonist	8.1	1.8	DYKDLCSQSGVGRIGWLACLPKK (ϵ -biotin)
D117	IGFR H2C	1	0.70	YES	-20 μ M	Agonist	6.1 5.1	8.6 8.5	FHENFYDWFVRQVSKK (ϵ -biotin)
D118	20E2	2	0.25	YES	-20 μ M	Antagonist	1.3	5.2	DYKDFYDAIDQLVRGSARAGTRDKK (ϵ -biotin)
D119	20C11	2	0.25	YES	-20 μ M	Antagonist	13 2.5	2.9 0.8	KORAFYNGLRDLVGAVYGAWDKK (ϵ -biotin)
D120	E8	10	0.37			Antagonist	2.2	5.9	KVRGFQGGTWPFGYEWLRNAKK (ϵ -biotin)
D121	F2	10	1.1			Antagonist	7.4	6.7	KSMFVAGSDRWPFGYGLADWLKK (ϵ -biotin)
D122	20A4 (A7)	6	1.2 1.0			Antagonist	>20 >20	>17 >20	KEIEAWGRVRLVYGRVCVGKK (ϵ -biotin)
D123	D8	6	0.55 1.3			Antagonist	16 >20	29 >15	KWLDOEWAWQCEYVGRGCPSSKK (ϵ -biotin)
D124	F8	4	0.04* 0.09*				8.2 >20	200 >200	KHLCLVEELFWGASLFGYCSGKK (ϵ -biotin)
D125	IGFR E4	1	2.6				>20	>8	DYKDESAAGFRGNFYDWFVAQVNNKK (ϵ -biotin)
D126	IGFR D2C	1	1.4				18	1.3	LGENFYDWFVMOVRKK

FIG. 5A

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Clonal Name	D or S name	Motif	Sequence	IR-Kd	IR-IC50 Biocore	IR-IC50FP-S175	PO4	Fat Cell Assay
20-E2	D118	B6	DYKDFYDAIDQLVRGSARAGGTRDK K-biotin	250 nM		2.8 nM	+	++
C1	D112	A6	DYKDCWARPCGDAANFYDFVQOAS KK-biotin	490 nM			-	0
D8	D123	C-C LOOP	KWLDQEWAWVQCEVYGRGCPSSK	550 nM			0	-
E8	D120	GROUP 6	KRGFGGTWPCYEWRNA	370 nM			-	-
F8	D124	C-C LOOP	KHLCLLELFWGASLFGYSGKK	40 nM			-	0
H2C	D117	A6	FHENFYDWRVROQSSK	700 nM	>5 μ M	5 nM	++	++
KCF9			RLYYEWFVQGLEAGRGGLS					
KC-C2		C-C-C	GLEGGCPWVGLVQCRCGPS					
KCG7		B6	PYCGLELSWGAALFGYSG		>1 μ M			
NG-C2		B6	GNCGMVFQLLSLVGRDMH					
NG-G33		A6	GHQSQCPESFYDWFAGQVSDPWVWCV		2-4 μ M	4.2 nM	+++	
NG-G8		B6	VEGRGLEFYDLLRQLARRONG		>5 μ M		-	
NG-G9		B6	RAMSFYDALVVLGLGPKK-Biotin				-	
RP-1		A6	CSRPFHEQFTLWFDQLGL	1 μ M			+	
RP-2		A6	RSEASFHVFYSWFEEQLRS	1 μ M			+	
RP-3		A6	GRFYGWFOADQLMPWGED	>10 μ M			-	
RP-4		B6	PPWGARYDAIEQLVFQNL	5 μ M			+	
RP-5		B6	AGVNAGFYRTFSTLLDWDQGGK-Biotin	6 μ M			-	
RP-6		B6+	IFYSCLASLLIGIPQPNRGPWERCCK-Biotin				+++++	
RP-7		C-C						
RP-7		A6	AAVHEQFYDWFADQYKK					
RP-8		B6	QSFYDIEELLGGEWKK	>5 μ M			+	
RP-8	S287	B6	QSFYDIEELLGGEWEE					
RP-9		A6	GSIDESFYDWFERQLGKK			2.9 nM	++	
RP-10		B6	GSFYEALORLVGGEQKK	>10 μ M			+	
RP-11		A6	QAPSNFYDWFVREWDKK	>10 μ M			+	
RP-12		B6	DPFYQGLWEWLRSGKK					
RP-13		A6	ASGFPENFYDWFGRQLSKK	>10 μ M				
RP-14		A6	SACOFDCHENFYDWFAROKK	>10 μ M				
RP-15		A6	SQAGSAFYAWFDQVLRVKK					
RP-16		B6	VMDARDDPFYHKLSELTCK					
RP-17		B6	QSDAFYSGLWALGLSDGKK	>10 μ M				
RP-18		B6	LQPCSGFYDWFQRIHLGSKK					
RP-19		A6	LKQGFYDWFQRIHLGSKK					
RP-20		B6	GSASFYDAIDRLRMRIKK					
RP-24		GROUP 6	WPGYLFEEALQWRGSTE					
S167	S167	A6	AFYDWF-AKK	>20 μ M	No Binding			
S173	S173	B6	LDALDRLMYEEERPSL	1.2 μ M				
S174	S174	B6	PLAELWAYFEHSEQGRSSAH	16 μ M				0
S175	S175	A6	GRVDWLORNAFYDWFVAELG	230 nM	2-4 μ M	0.9 nM	++	0
S176	S176	A6	NGYERAGIGDNFYDWFVAOLH	470 nM				+++

FIG. 5B

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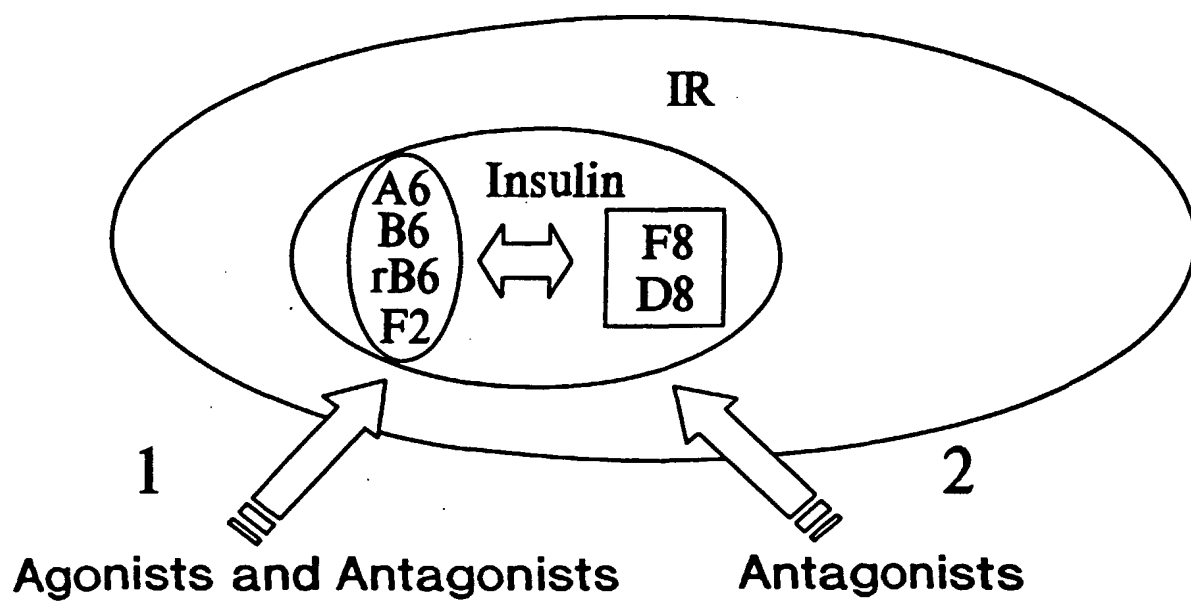


FIG. 6

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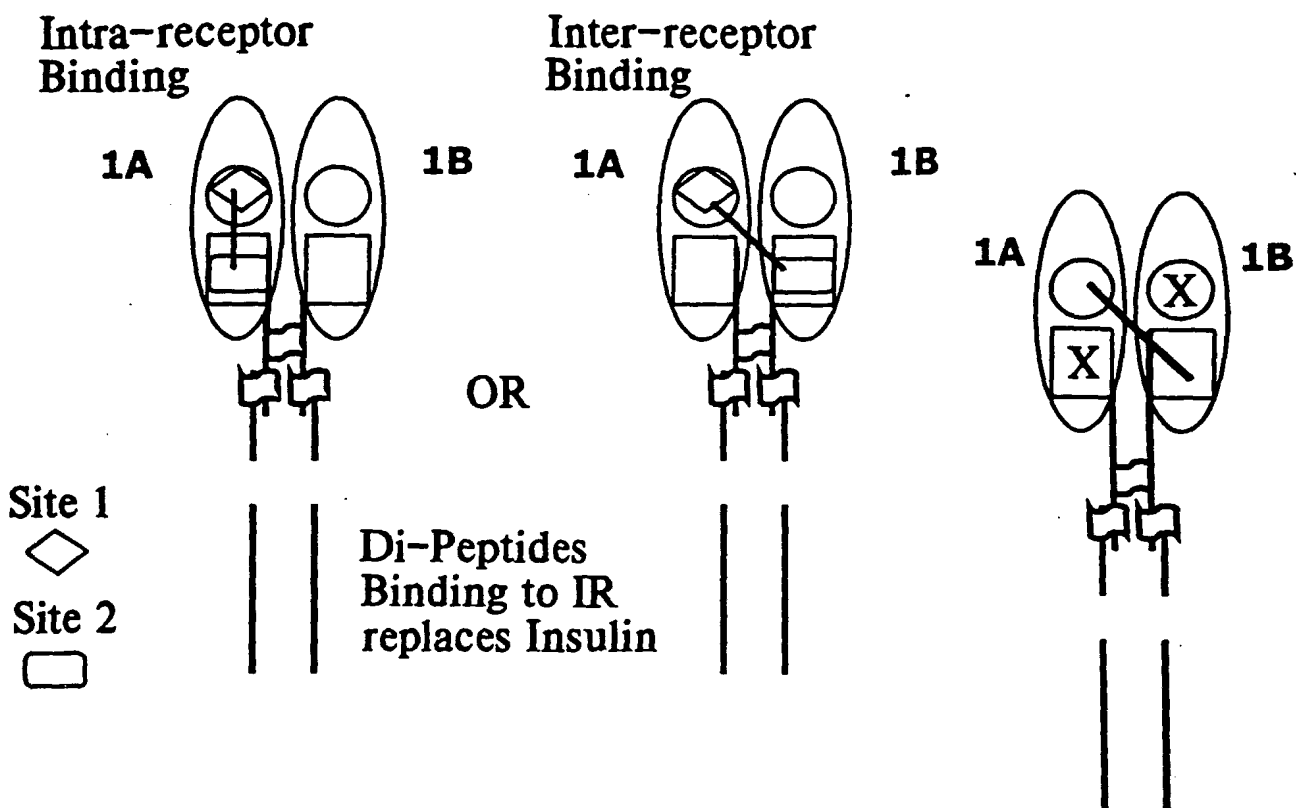


FIG. 7

Group 1 (Formula 1 Motif)		Target		
		Found	IR	IGF
20D3*	IGGQGHQDGNFYDWFVEALA	18	+	++++
20F1	VFWNCRSQQLDFYEWEEQAA	16	+	++++
G3	RGGGTFYEFESALRKHGAG	8	+	+++
20H1	RVAGAISAPGLVSNKQDGLFYSWFRE	5	+	+++
20D1*	VLQARHGCDVSVDCFYEWFA	4	+	++++
D2	PERMQSDVGFYEFWFRAAVG	3	+	+++
B8	WSALLSVMDTGEYAWFDDAV	2	++	++++
C4	DIGSDGHGRRWDSFYRWFEEM	2	+	+++
A8	IGGSFVEFYGFENDQV	2	+	+++
E7	GHSWALVRHVDRRLFYEWFDL	1	++	+++
C8	LPAGGAQGFVAVRGFYEFWFS	1	+	+++
H8	RDKPTDQEEQNWVSFYEFWRH	1	+	+++
E2	SRDQTNFTFNSAGFYGFWFER	1	+	+++
B12	GAFYRWFHEALVGSERVPDV	1	+	+++
D10-2	RIGGGWARSEGEFYEFVREL	1	+	++
G8	RMFYEFWFSQMGAGPTEGSA	1	+	++
H3	HEAFYDWFESALVDGGYELMG	1	+	++
3G11	FYGFWSRQLSLTPRDDWGLP	1	+	++
F4	GVGTLTMSSDAFYTWEV	1	+	++
E7-2	LGTSAGQGVGHRAFYQWFQS	1	+	+
40G11	<---ETLESHYVVPQ-----AALDRLFYSWFS	3	+	++++
40B2	IRDMHYVWVQDRDRYINGVRQWYISDRYNPGSAFYRWFEID	2	+	++
40B12	RMGLQALAHYRKSA-----GPIFLSSGSVIKGSEGDPFYAWFRLO	1	+	++

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FIG. 8

Target		
Found	IR	IGF
13	+++	0
3	+++	?

Group 2: Formula 6 Motif

20A4* EIEAEWGRVRC¹LVYGRCVGG
D8 WLDQEWAWVQCEVYGRGCP²S

Target		
Found	IR	IGF
1	+	+++
1	+	+++
1	+	+++
1	+	+++
1	+	+++
1	+	0

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Group 3: Formula 2 Motif

20E2 DYKDFYDAIDQ¹LVRSARAGGTRD
20C11 DYKDDRAFYNG¹LRDLVGAVYGAWD
20A12 DYKDRLEFYCGIQ¹ALGANLGYSGCV
C6 DYKDFYSALW¹GLCGVTGCG
A6 RGQSDAFYSG¹LWALIGLSDG
40H4 RYFPFGGFTYGN¹LDVLRWLRPYVASPRWGHWRPGSLGKQPT

Target		
Found	IR	IGF
1	++	++
1	+	+

Group 5: Miscellaneous Motif 10

D9-2 PFGFGGRWWGI¹PRMWWYRNS
H4 WWWGGRNRWW¹LERWGLGGER

FIG. 9A

		Target	
Found		IR	IGF
3		++	0
1		++	+
1		++	++
1		++	?
1		0	+++

Group 4 and 6: Miscellaneous Motif 10

D10 LGPLLRLWGSEVCGVWPDICE
A2 GRVALWGPVWPRWWFMSRPV
F2 SMFVAGSDRWPYGVGLADWL
E8 VRGFQGGTVWPGYEWLRNAA
A4 WPGYLFFEEALQDWRGSTED

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		Target	
Found		IR	IGF
1		0	++
4		+++	+
1		0	0

Group 7: Formula 4 Motif

B6 ACSSEFFVKPEGEFLQCLGSI
F8 HLCVLEELFWGASLFGYCSG
40D6 PERGRGLRTAMQLMRRPRDWHFPHSLFWGAPPLSG

FIG. 9B

FIG. 10A

Displacement of ¹²⁵I-HI from HIR-11

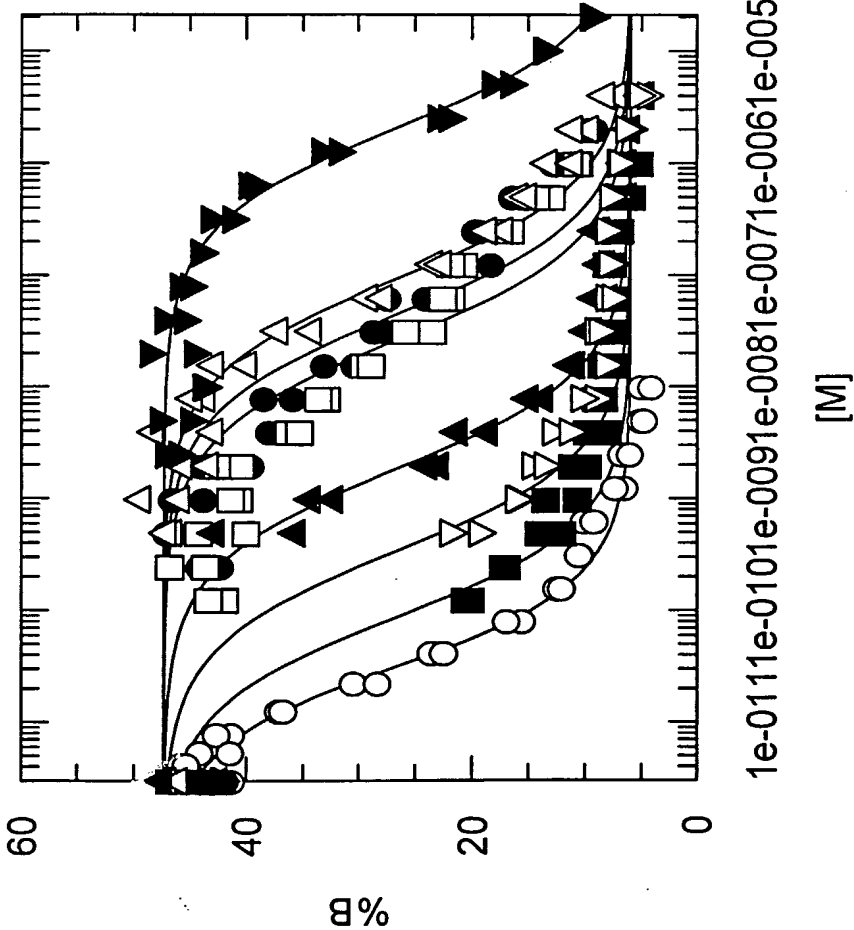


FIG. 10B

FIG. 10C

FIG. 11A

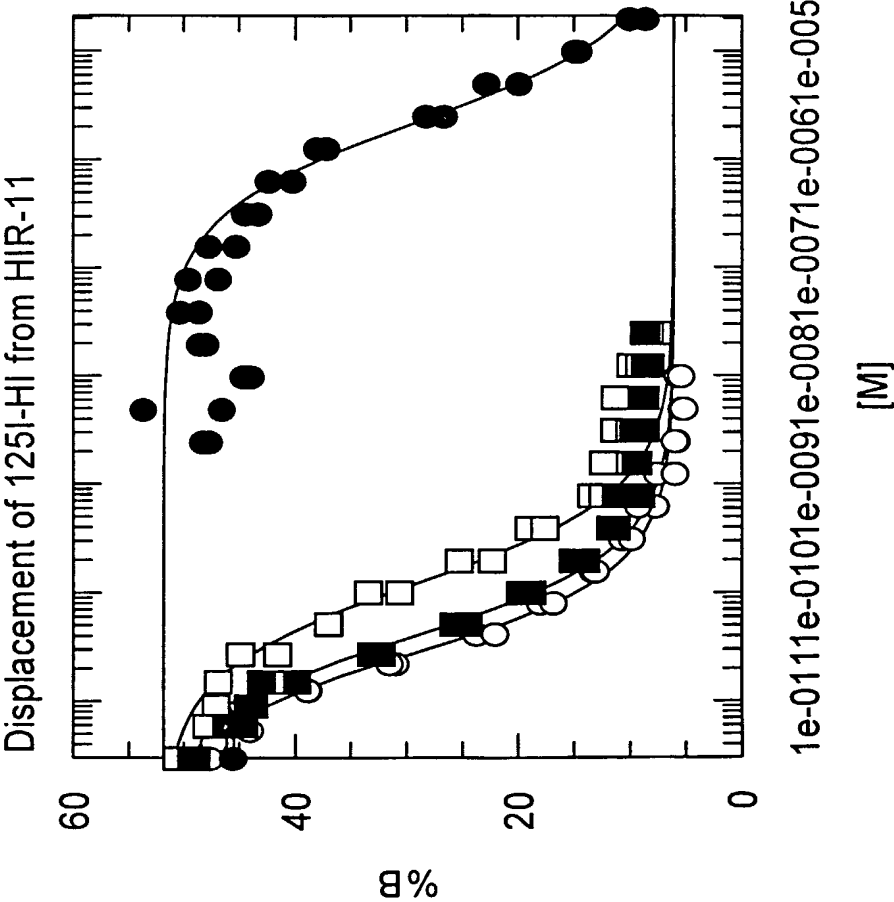


FIG. 11B

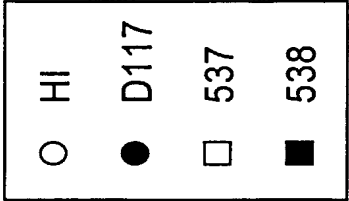
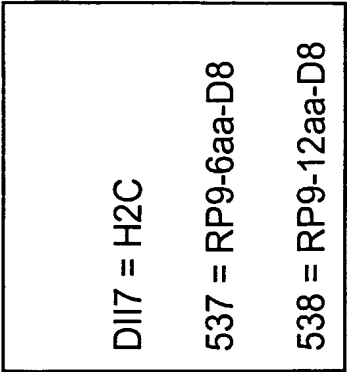


FIG. 11C



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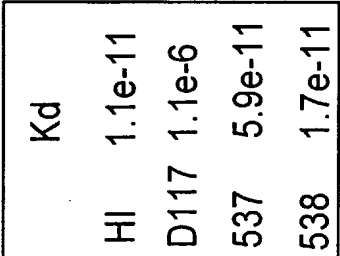


FIG. 11D

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FIG. 12B

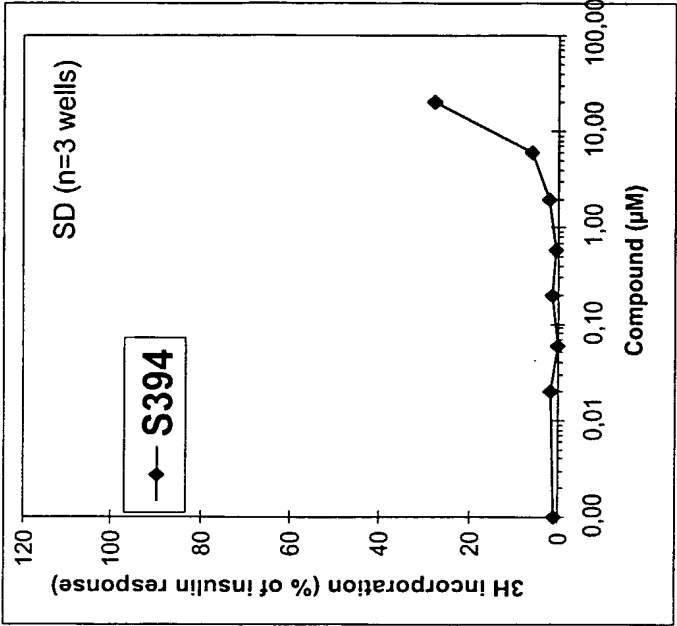
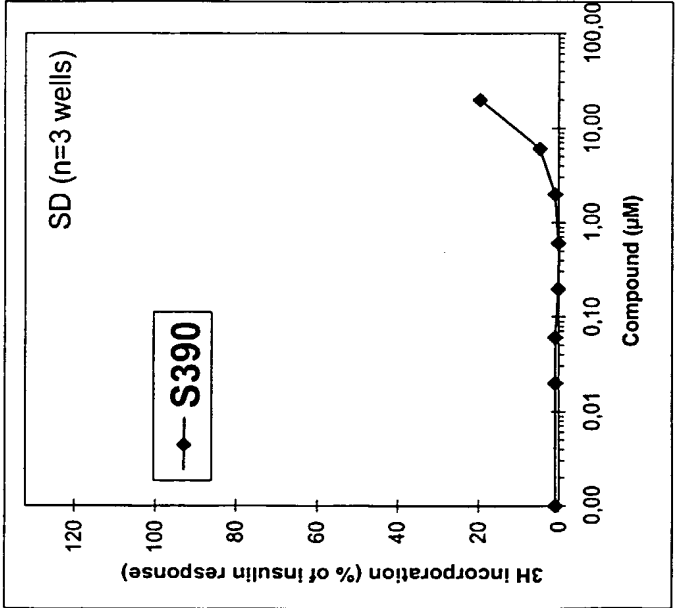


FIG. 12A



S390 = ESFYDWFERQLG
S394 = GSLDESFYDWFERQ

FIG. 12C

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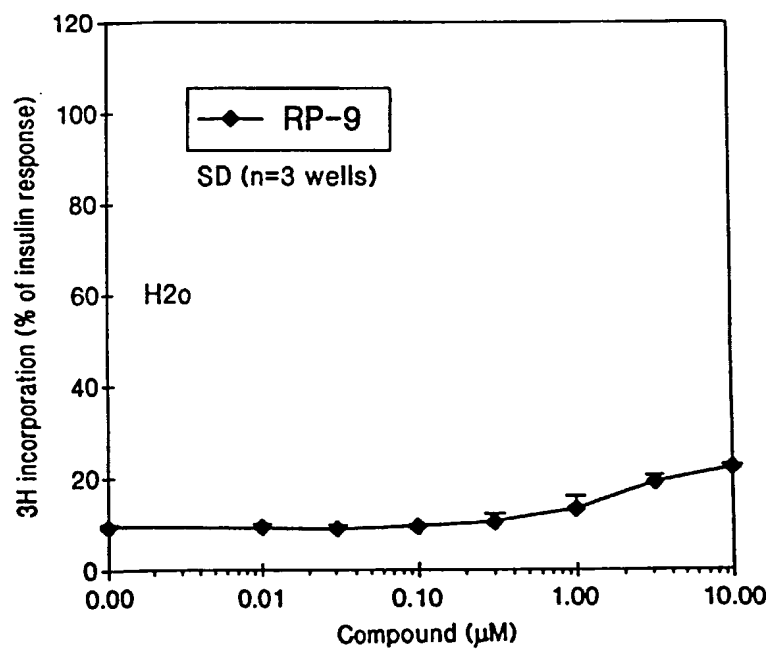


FIG. 12D

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FIG. 13B

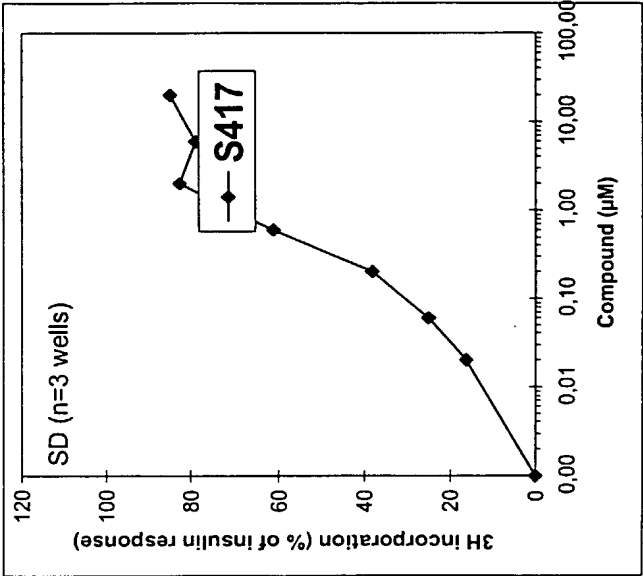
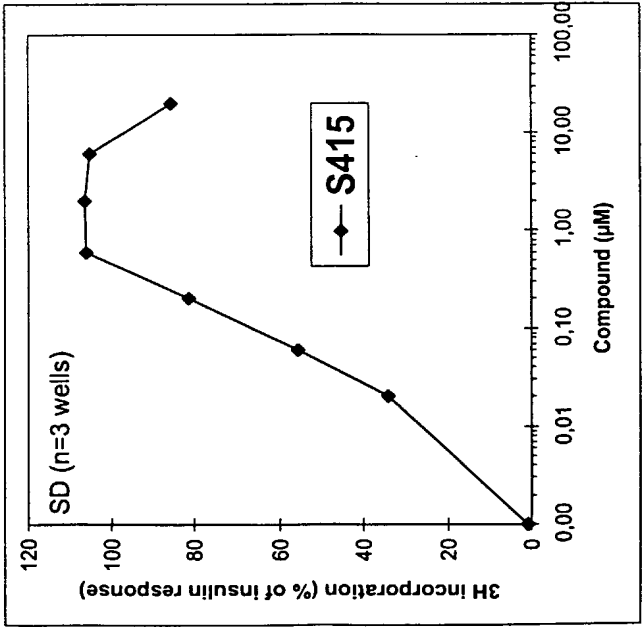


FIG. 13A



S415	(ESFYDWFERQLGK) ₂ - <u>23</u>
S417	<u>23</u> -(ESFYDWFERQLG) ₂

FIG. 13C

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FIG. 14B

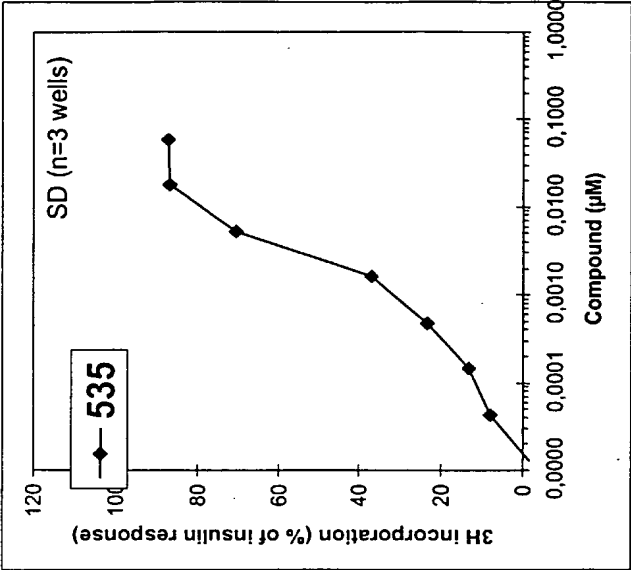
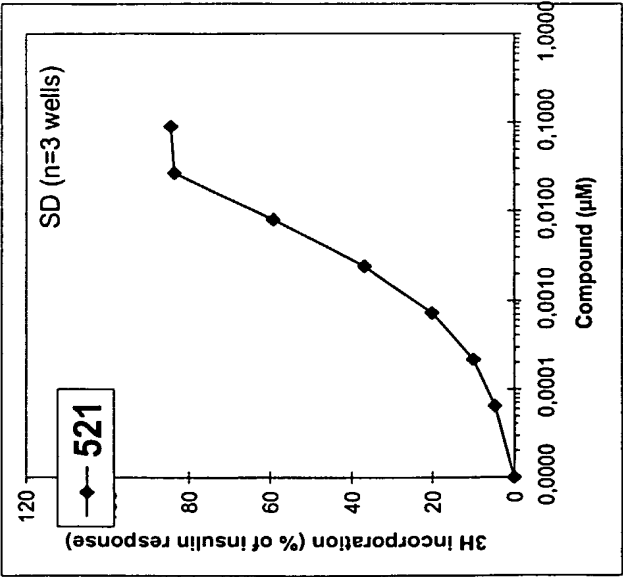


FIG. 14A



521 = RP9-6aa-RP9
535 = RP9-12aa-RP9

FIG. 14C

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FIG. 15B

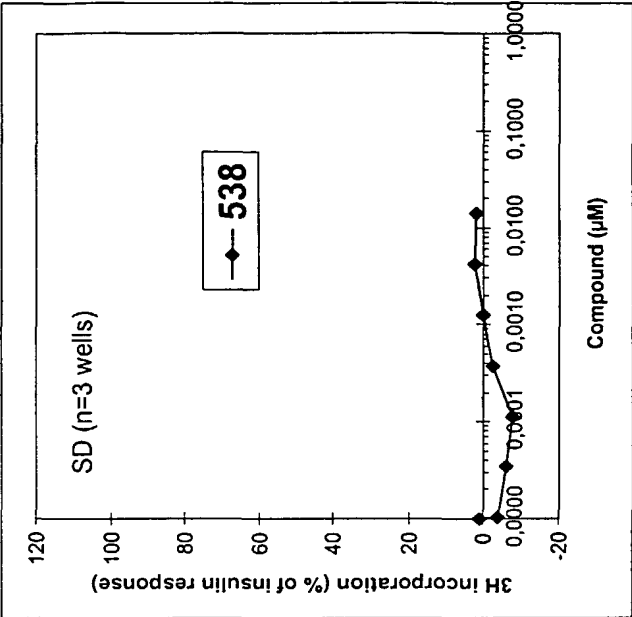
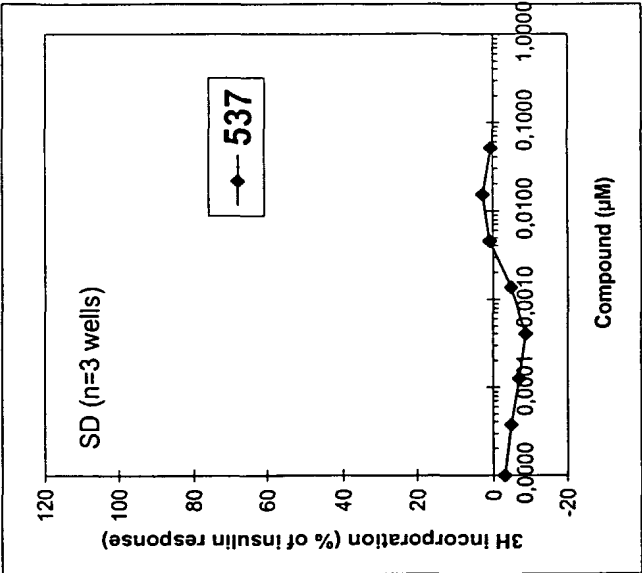


FIG. 15A



537 = RP9-6aa-D8
538 = RP9-12aa-D8

FIG. 15C

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FIG. 16B

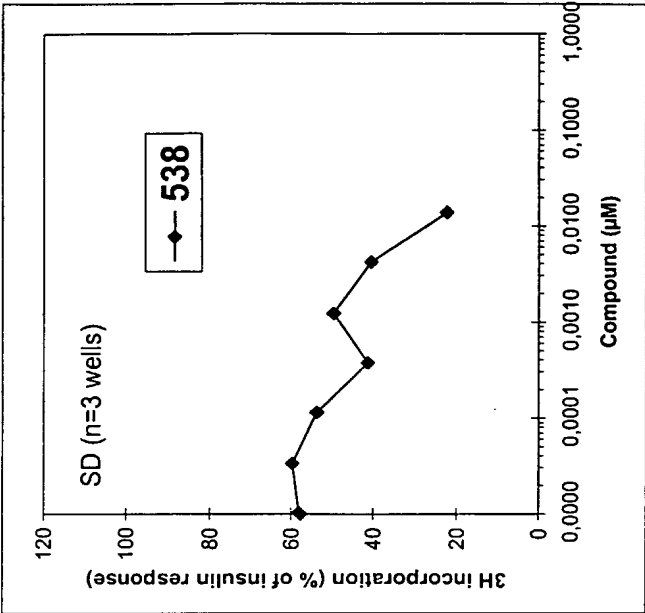
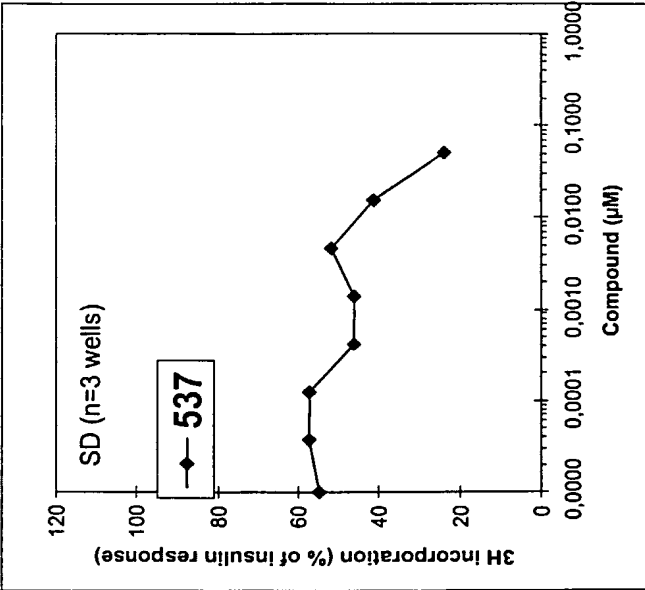


FIG. 16A



537 = RP9-6aa-D8
538 = RP9-12aa-D8

FIG. 16C

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FIG. 17A

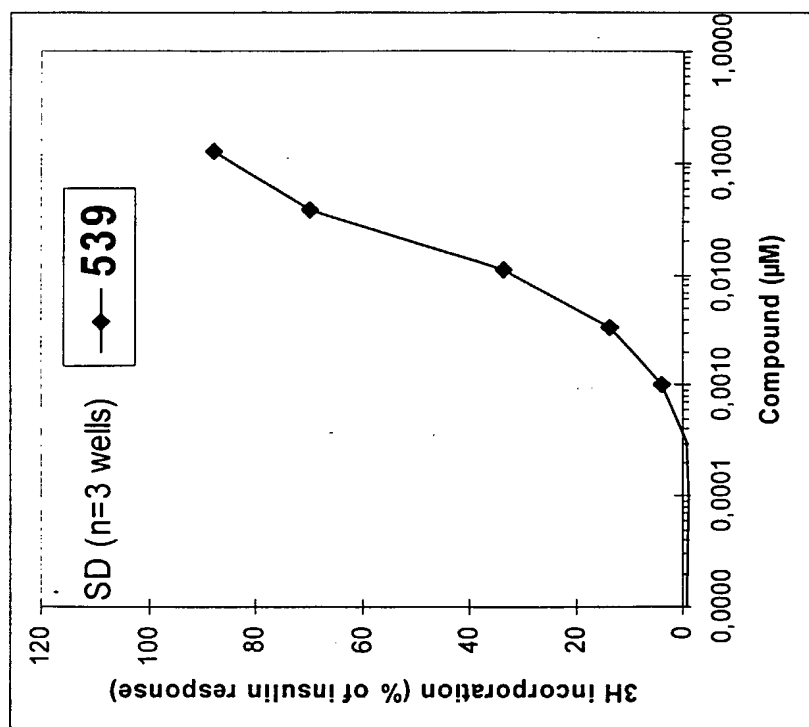
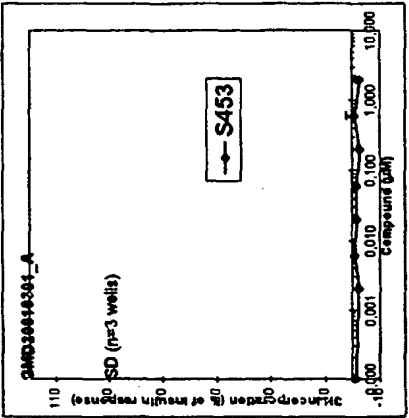


FIG. 17B

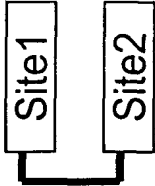
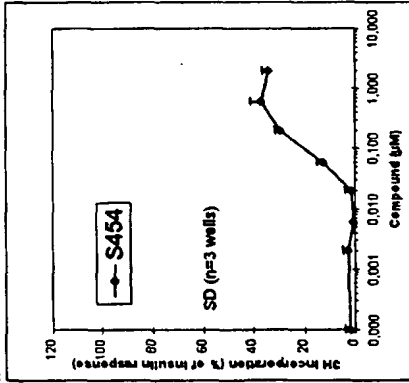
539 = D8-6aa-RP9

FIG. 18A



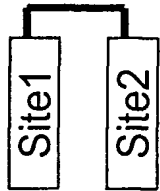
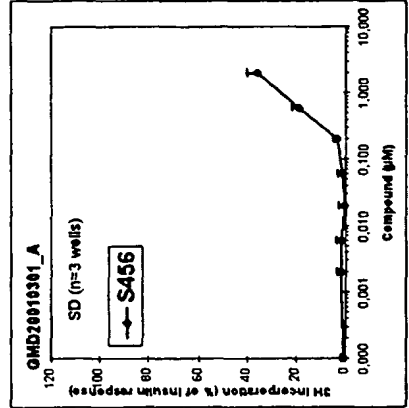
C-N

FIG. 18B



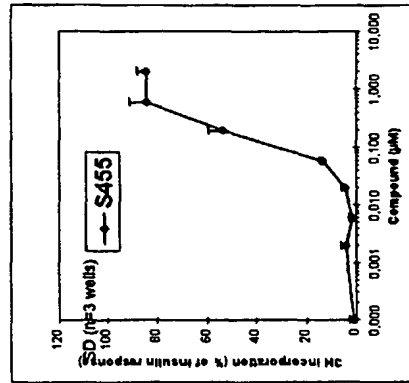
N-N

FIG. 18C



C-C

FIG. 18D



N-C

FIG. 19A

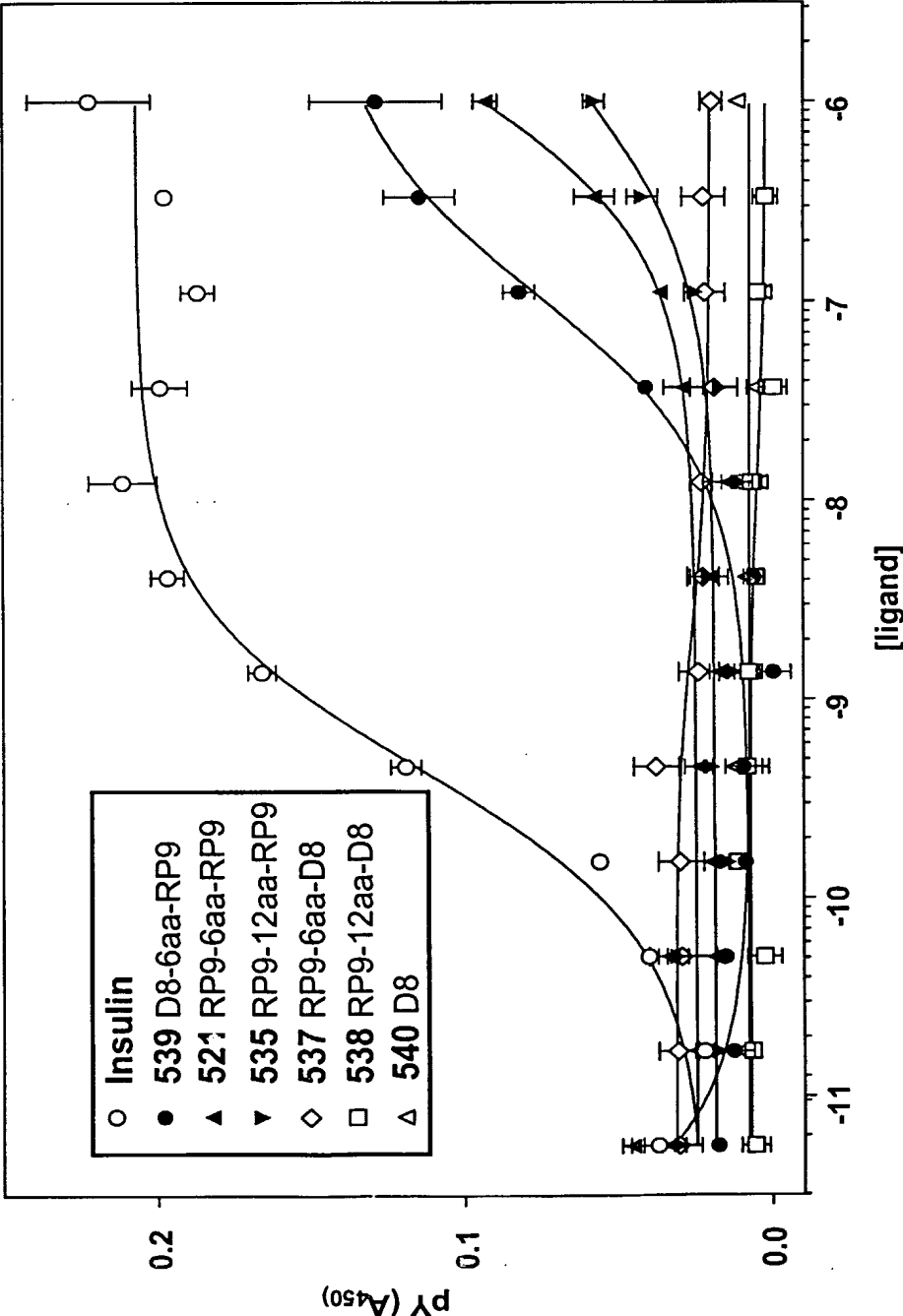
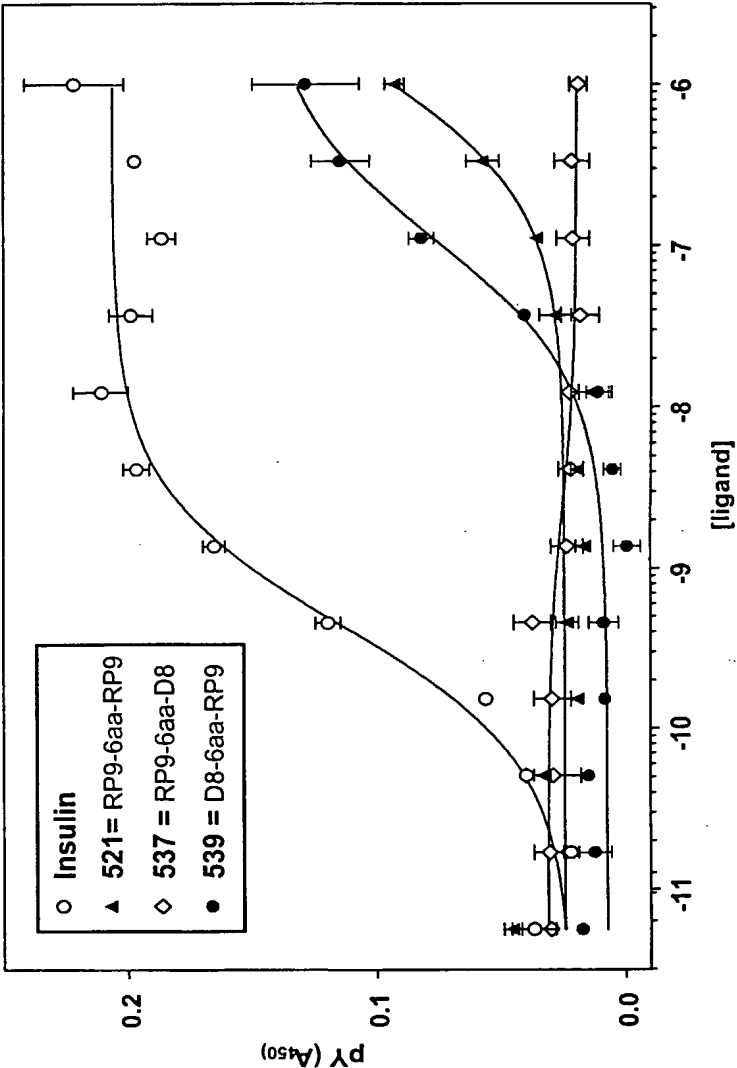


FIG. 19B

EC50	Insulin	521	535	539
	4.4680e-010	1.4420e-006	9.6490e-007	1.1000e-007

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FIG. 20A



	Insulin	521	539
EC50	4.4690e-010	1.4420e-006	1.1000e-007

FIG. 20B

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FIG. 21A

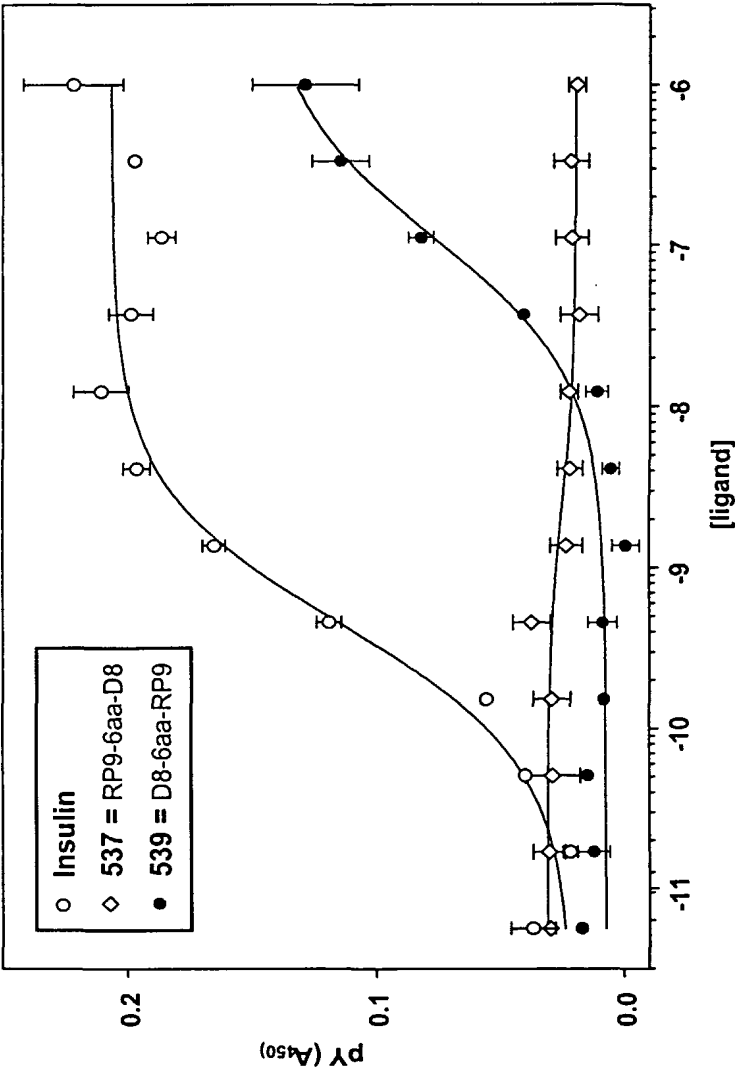


FIG. 21B

	Insulin	539
EC50	4.4680e-010	1.1000e-007

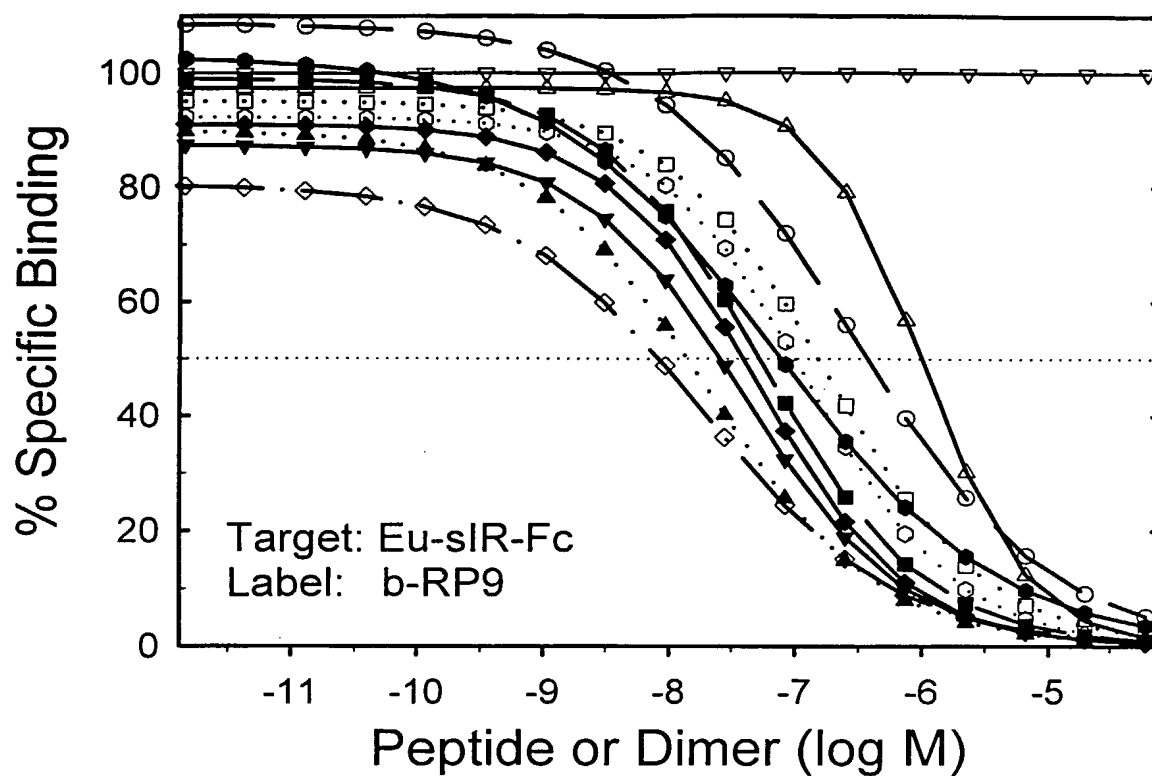
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FIG. 22A

FIG. 22B

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FIG. 23A

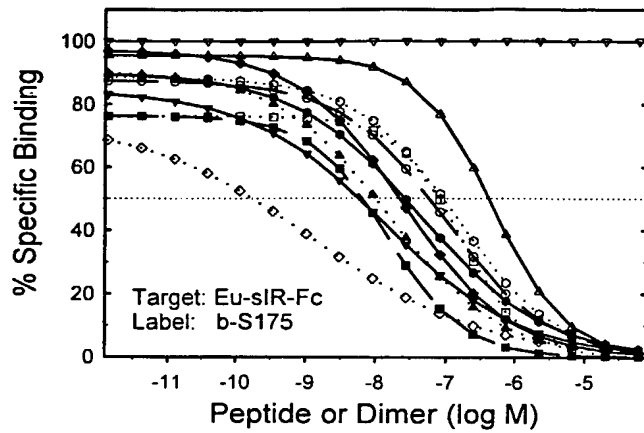
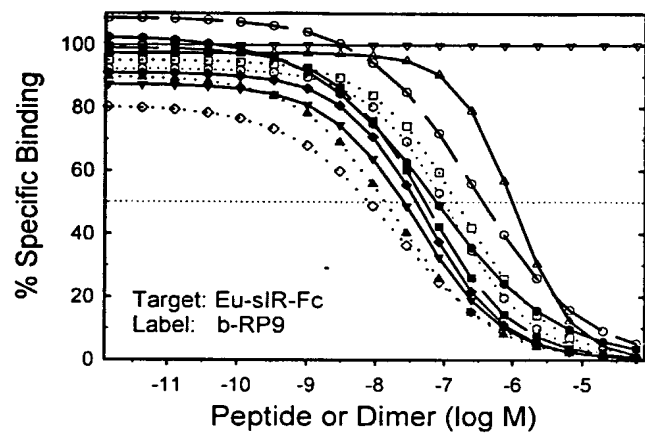


FIG. 23B

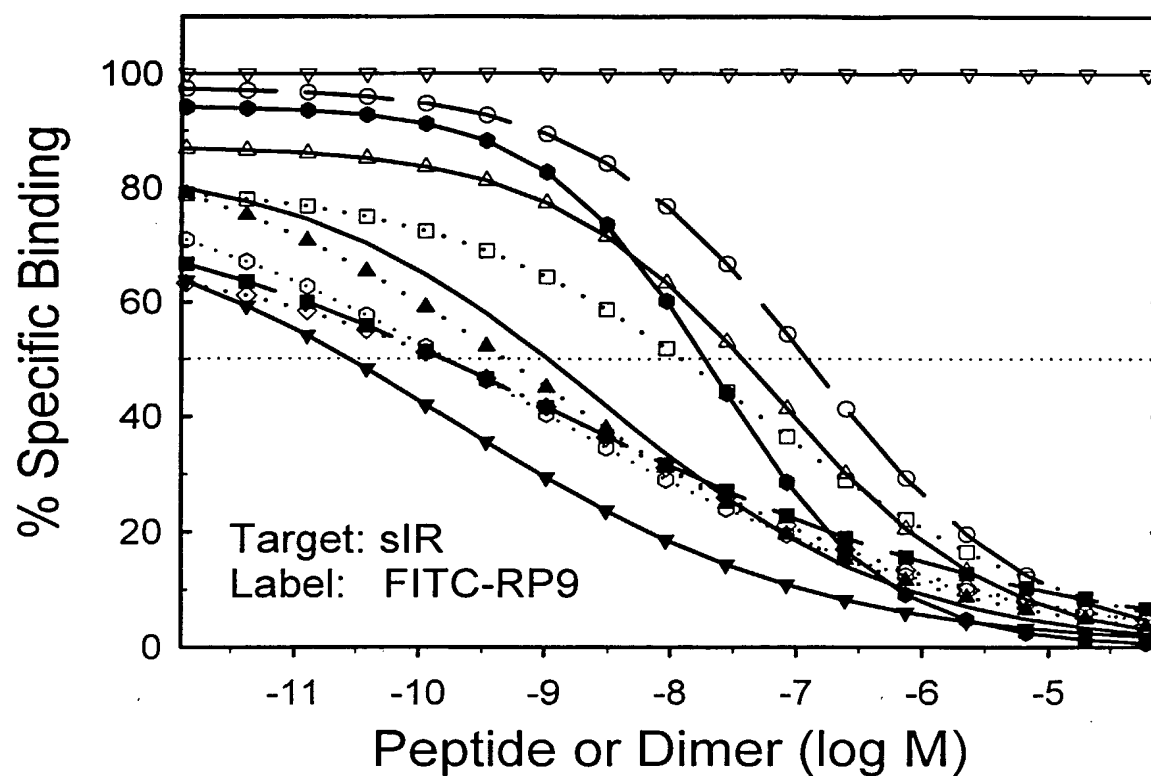


Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFVQRVSKK
□	S291	(Lig-GGG-H2C) ₂₋₉
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)- <u>14</u> -(RP9-Lig)
◇	S337	(RP9-Lig) ₂₋₂₃
○	S391	truncated(-GSLDE)RP9(-KK)
●	S390	truncated(-GSLD)RP9(-KK)
○	S414	(truncated(-GSLD)RP9(-KK)) ₂₋₁₄
◆	S175	GRVDWLQRNANFYDWFVAELG
▲	S380	(EE-short-S175-Lig) ₂₋₉
▽	Linker <u>23</u>	

FIG. 23C

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FIG. 24A



Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFVQRVSKK
□	S291	(Lig-GGG-H2C) ₂₋₉
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)- <u>14</u> -(RP9-Lig)
◇	S337	(RP9-Lig) ₂₋₂₃
○	S391	truncated(-GSLDE)RP9(-KK)
●	S390	truncated(-GSLD)RP9(-KK)
○	S414	(truncated(-GSLD)RP9(-KK)) ₂₋₁₄
◆	S175	GRVDWLQRNANFYDWFVAELG
▲	S380	(EE-short-S175-Lig) ₂₋₉
▽	Linker <u>23</u>	

FIG. 24B

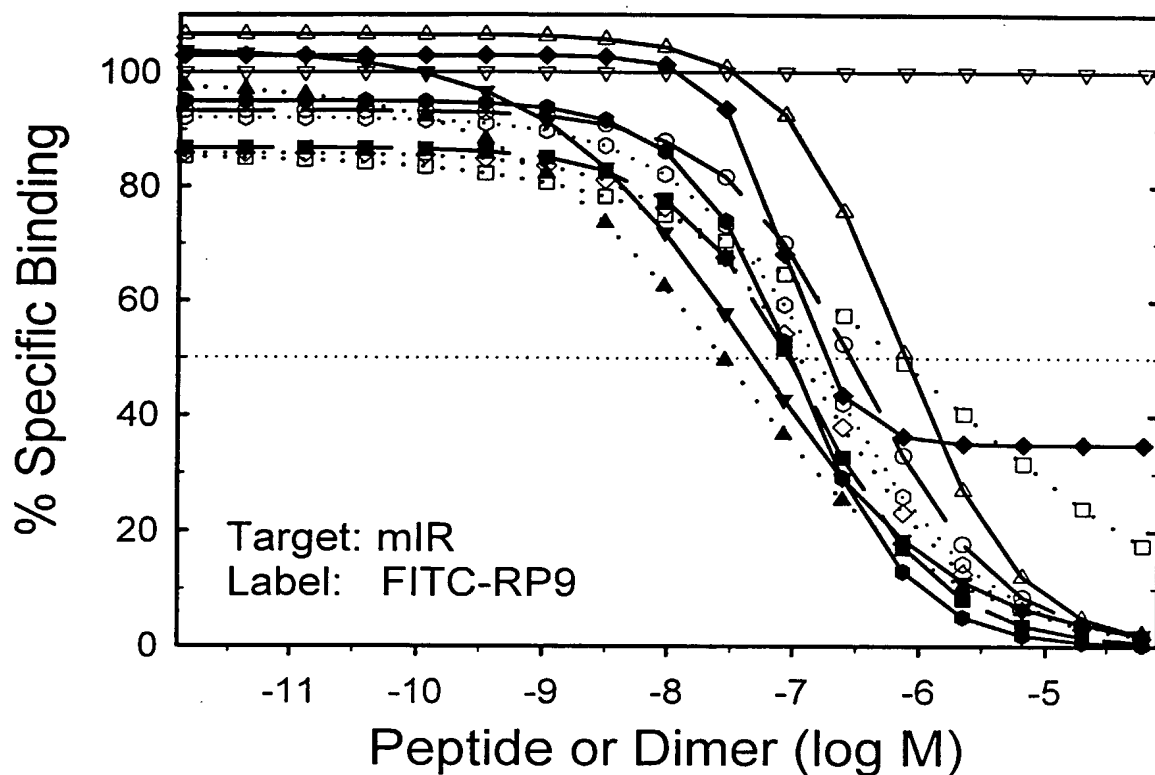
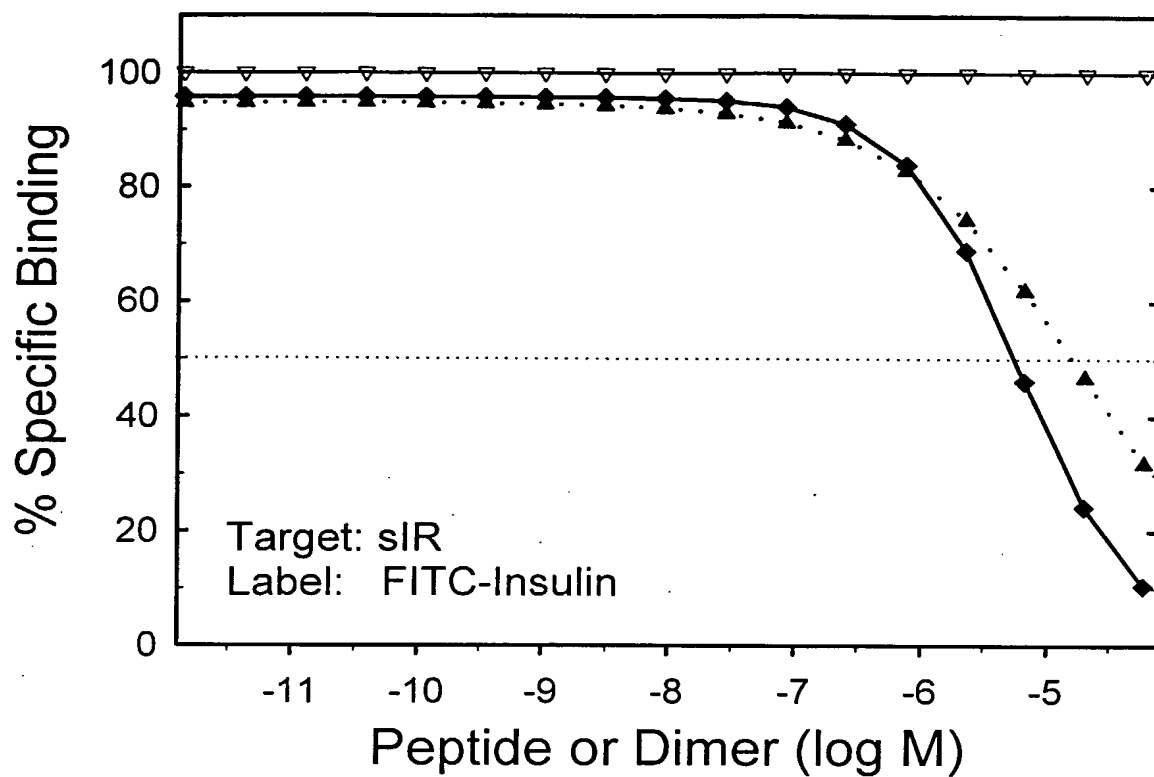
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FIG. 25A

FIG. 25B

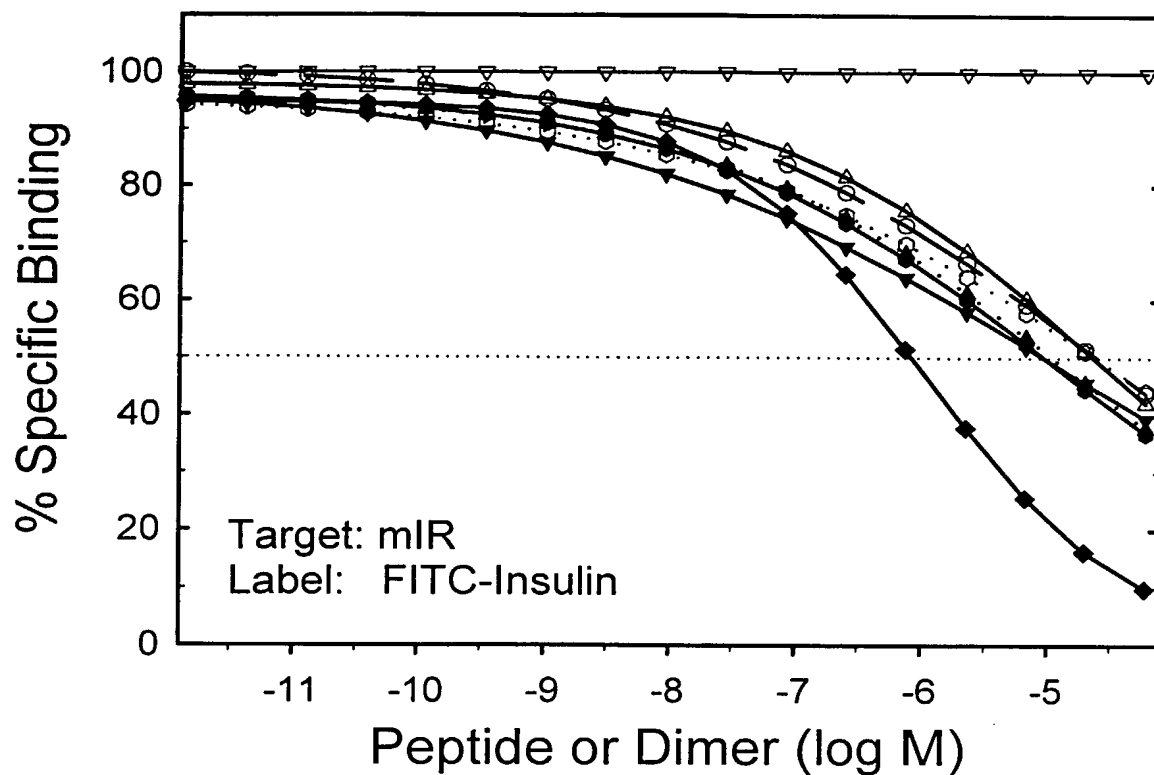
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FIG. 26A



Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFVQRVSKK
□	S291	(Lig-GGG-H2C) ₂₋₉
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)- <u>14</u> -(RP9-Lig)
◇	S337	(RP9-Lig) ₂₋₂₃
○	S391	truncated(-GSLDE)RP9(-KK)
●	S390	truncated(-GSLD)RP9(-KK)
◊	S414	(truncated(-GSLD)RP9(-KK)) ₂₋₁₄
◆	S175	GRVDWLQRNANFYDWFVAELG
▲	S380	(EE-short-S175-Lig) ₂₋₉
▽	Linker <u>23</u>	

FIG. 26B

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FIG. 27A



Symbol	Peptide or Dimer	Sequence
△	H2C	FHENFYDWFVQRVSKK
□	S291	(Lig-GGG-H2C) ₂₋₉
▼	RP9	GSLDESFYDWFERQLGKK
■	S375	(RP9-Lig)- <u>14</u> -(RP9-Lig)
◇	S337	(RP9-Lig) ₂₋₂₃
○	S391	truncated(-GSLDE)RP9(-KK)
●	S390	truncated(-GSLD)RP9(-KK)
◊	S414	(truncated(-GSLD)RP9(-KK)) ₂₋₁₄
◆	S175	GRVDWLQRNANFYDWFVAELG
▲	S380	(EE-short-S175-Lig) ₂₋₉
▽	Linker <u>23</u>	

FIG. 27B

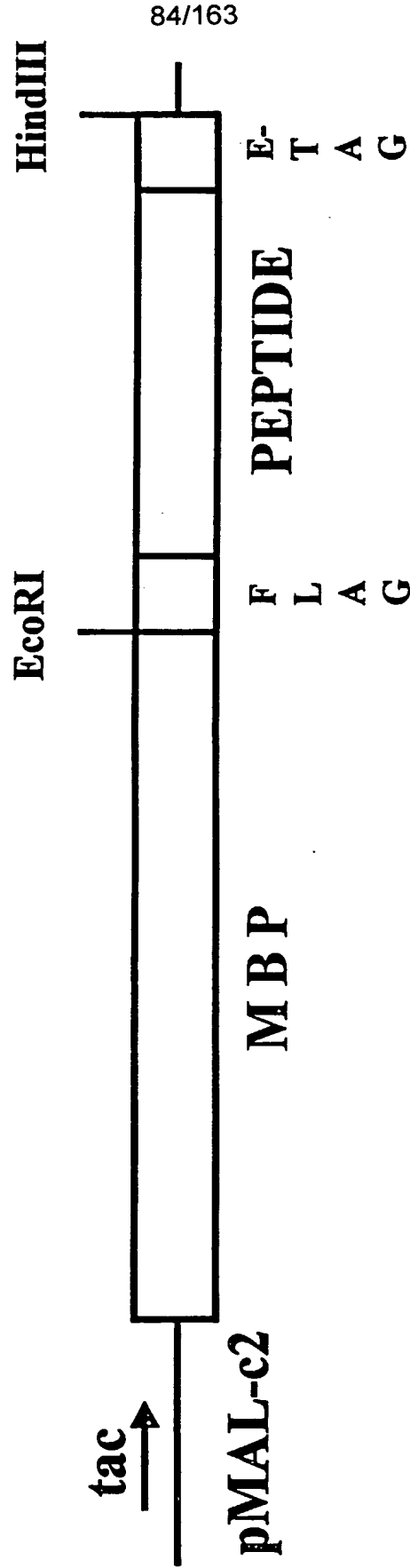
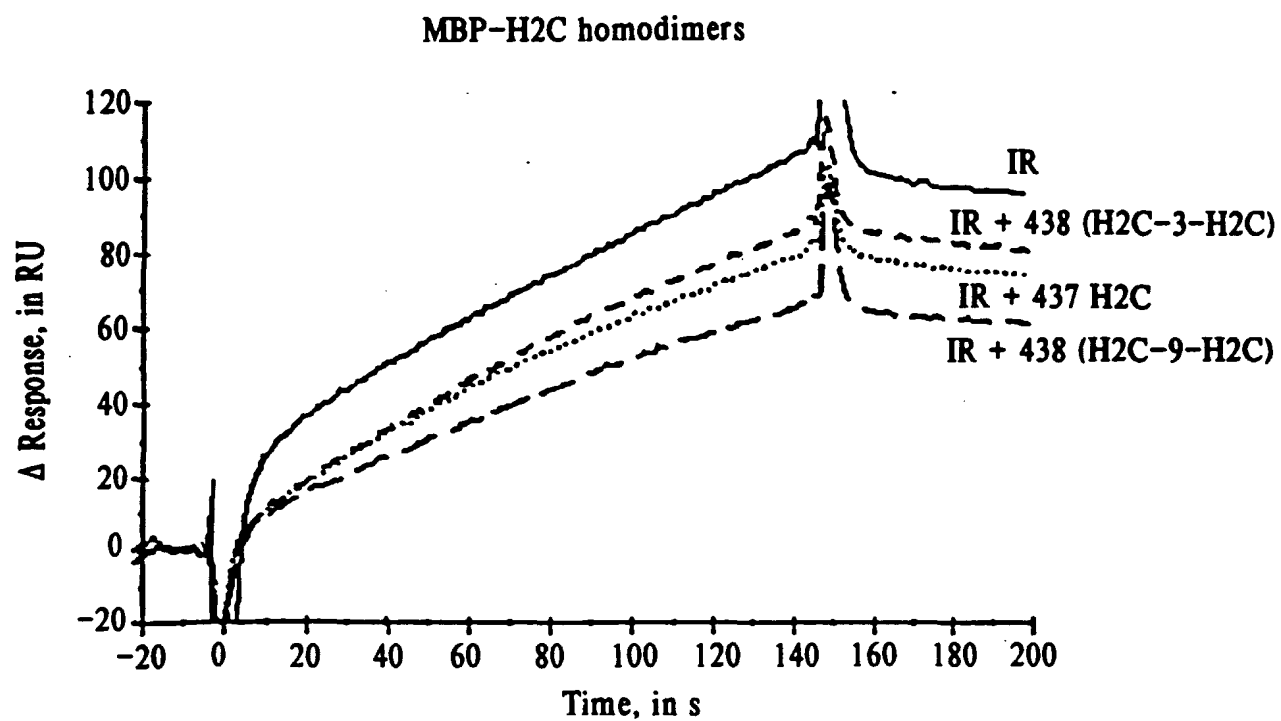


FIG. 28

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**FIG. 29**

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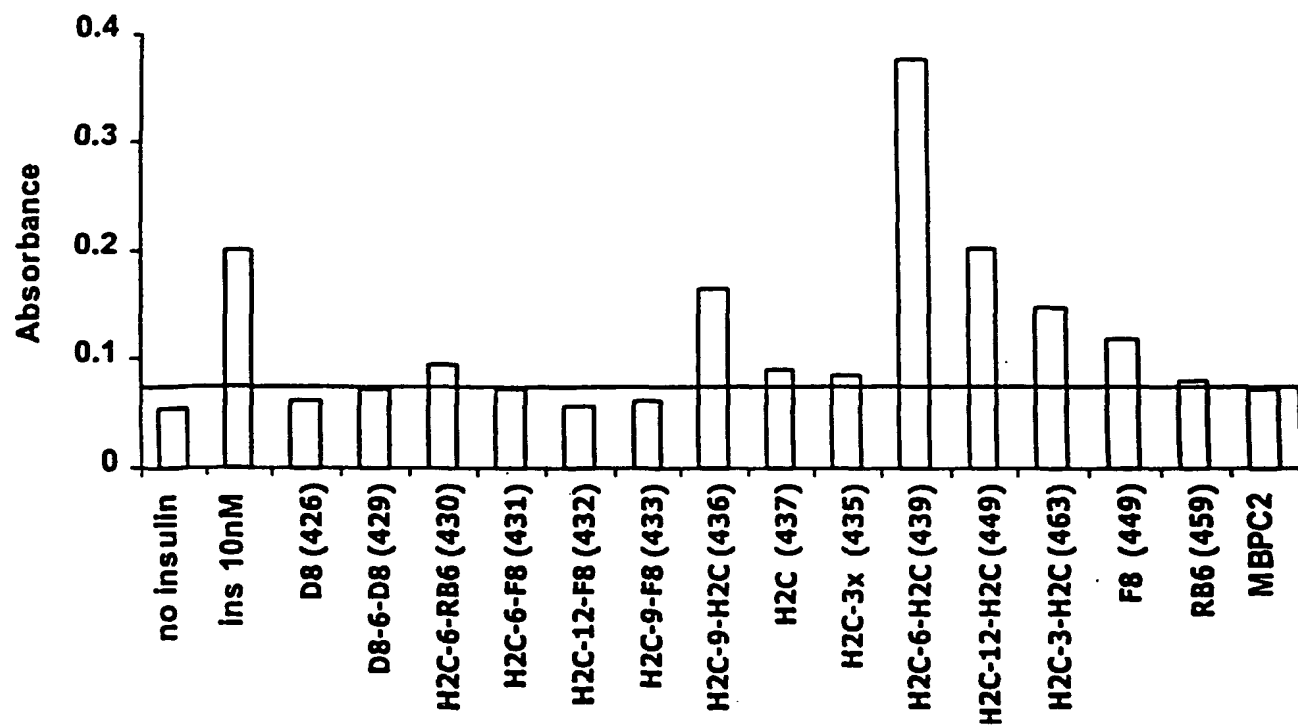


FIG. 30

FIG. 31B

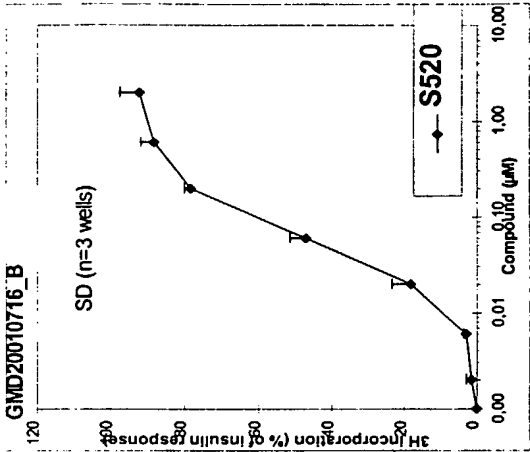
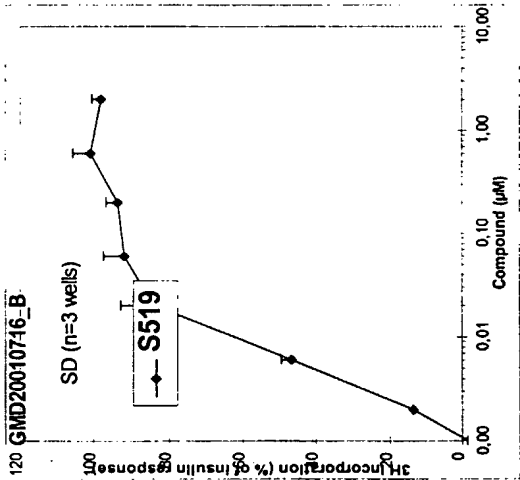


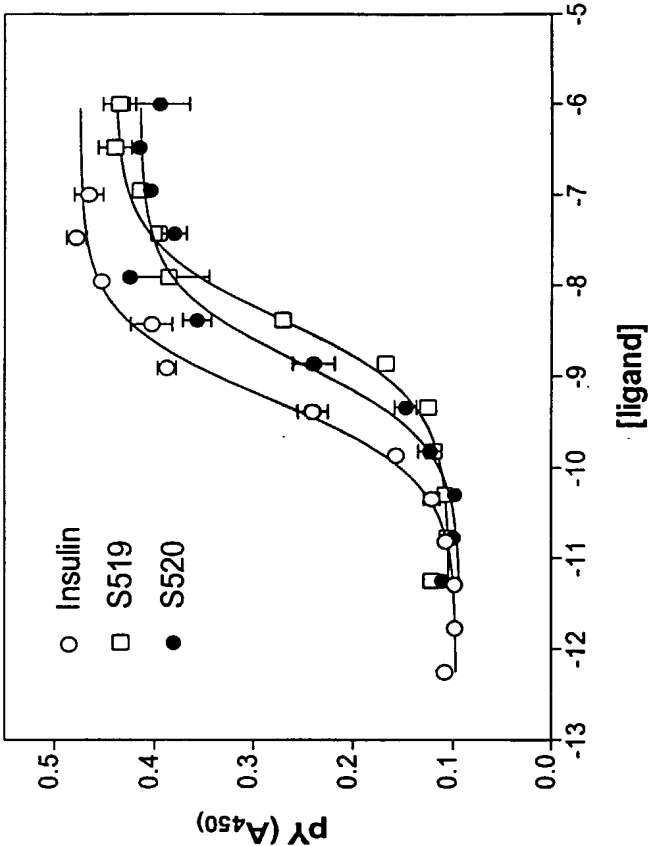
FIG. 31A



EC ₅₀	
■ Insulin:	0.050 nM
S519:	4.19 nM
S520:	58.8 nM

FIG. 31C

FIG. 32A



Equation 1	Insulin	S519	S520
Best-fit values			
BOTTOM	0.09614	0.1038	0.09202
TOP	0.4740	0.4388	0.4145
LOGEC50	-9.237	-8.380	-8.852
EC50	5.8000e-010	4.1660e-009	1.4060e-009

FIG. 32B

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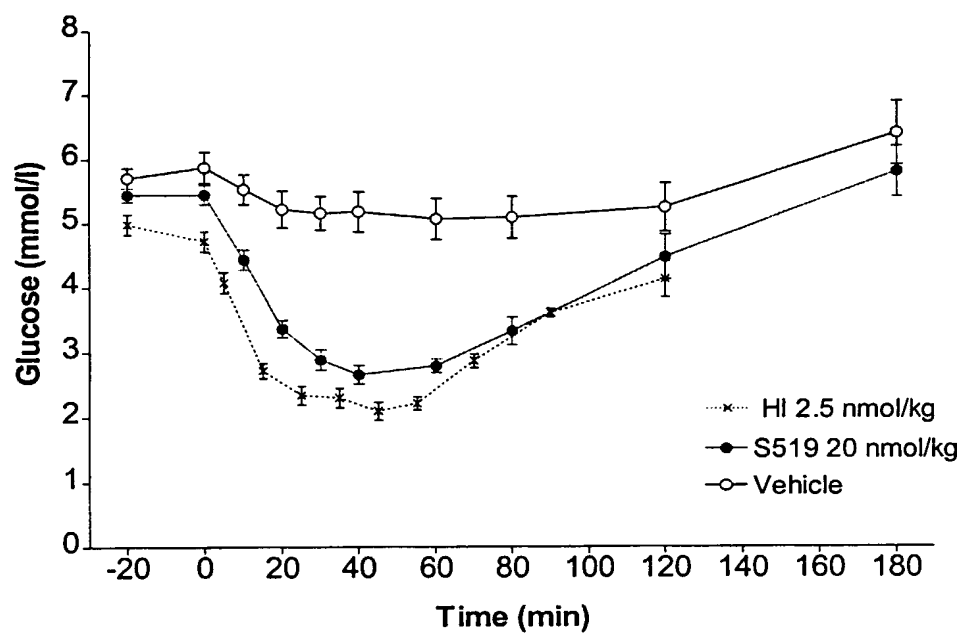
FIG. 33

FIG. 34A

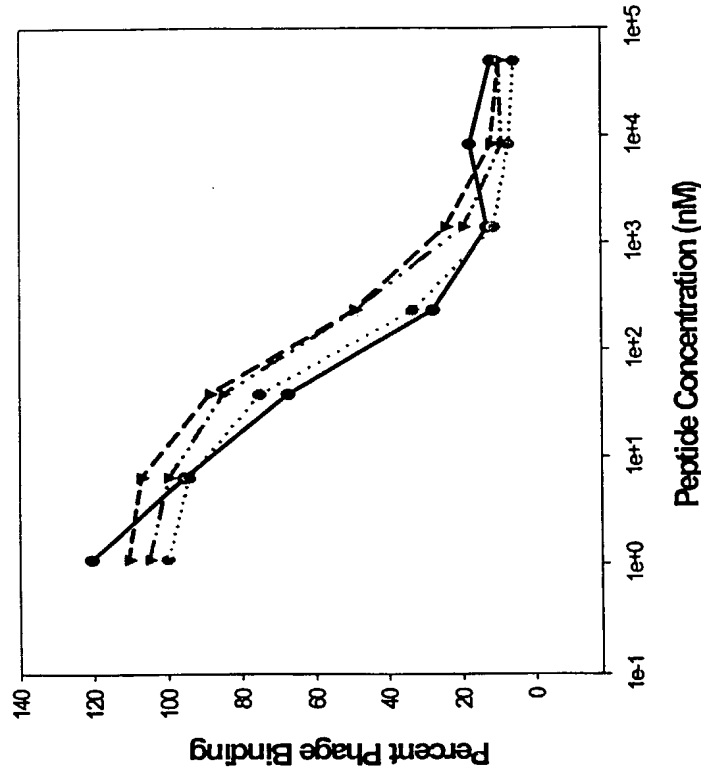


FIG. 34B

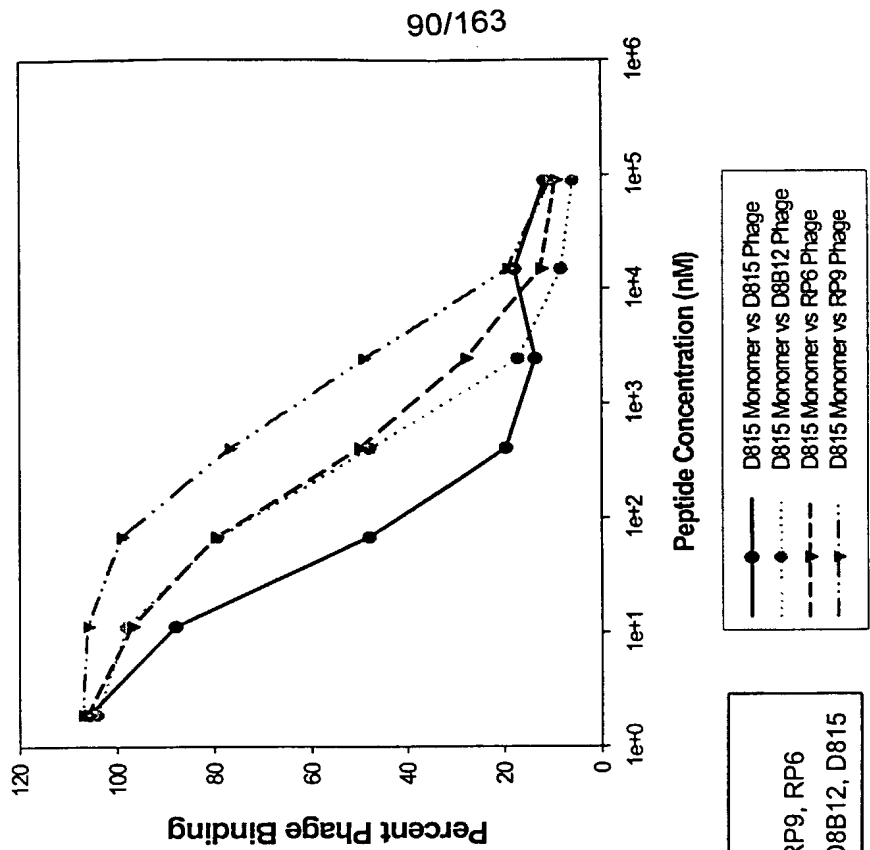


FIG. 34C

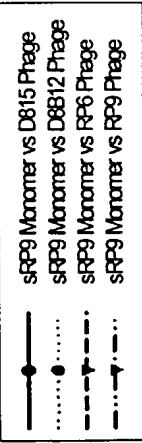


FIG. 34D

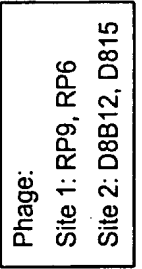


FIG. 34E

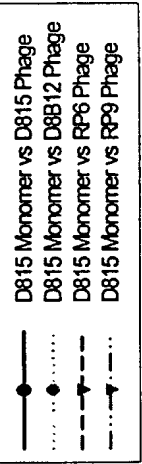


FIG. 35A

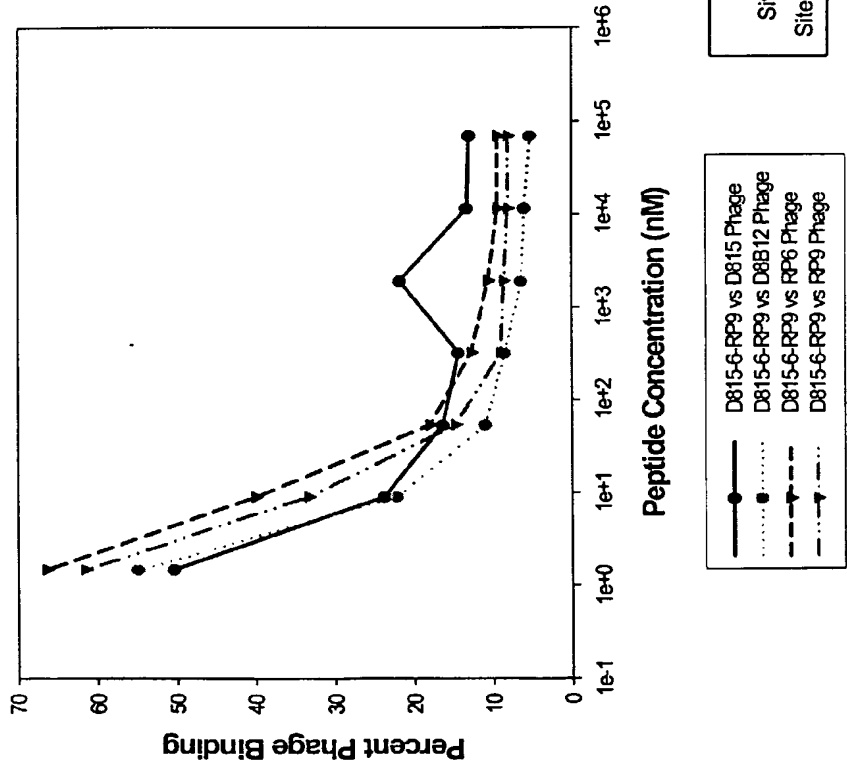


FIG. 35C

FIG. 35B

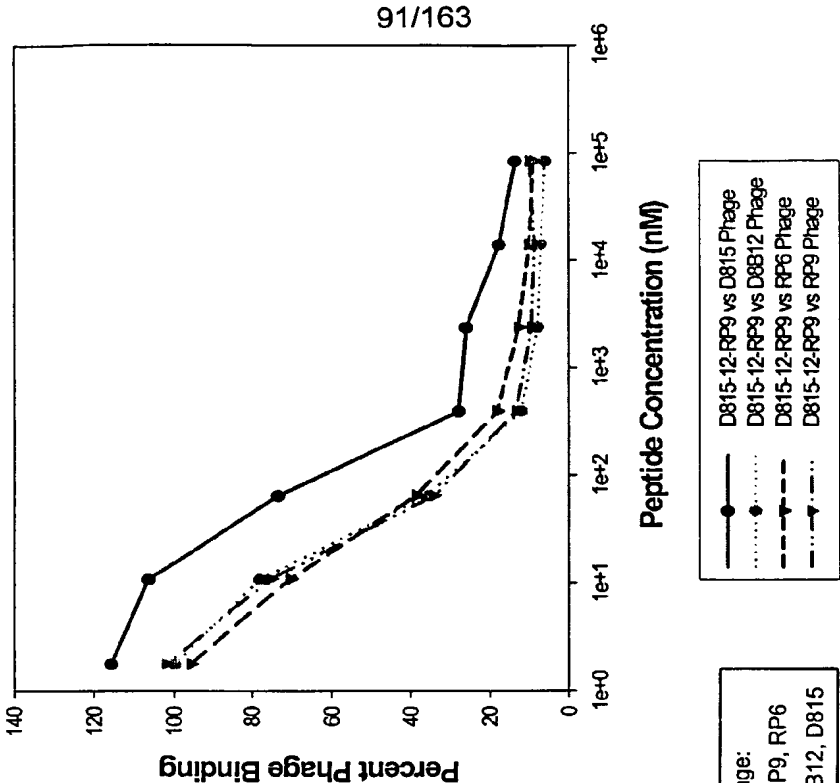


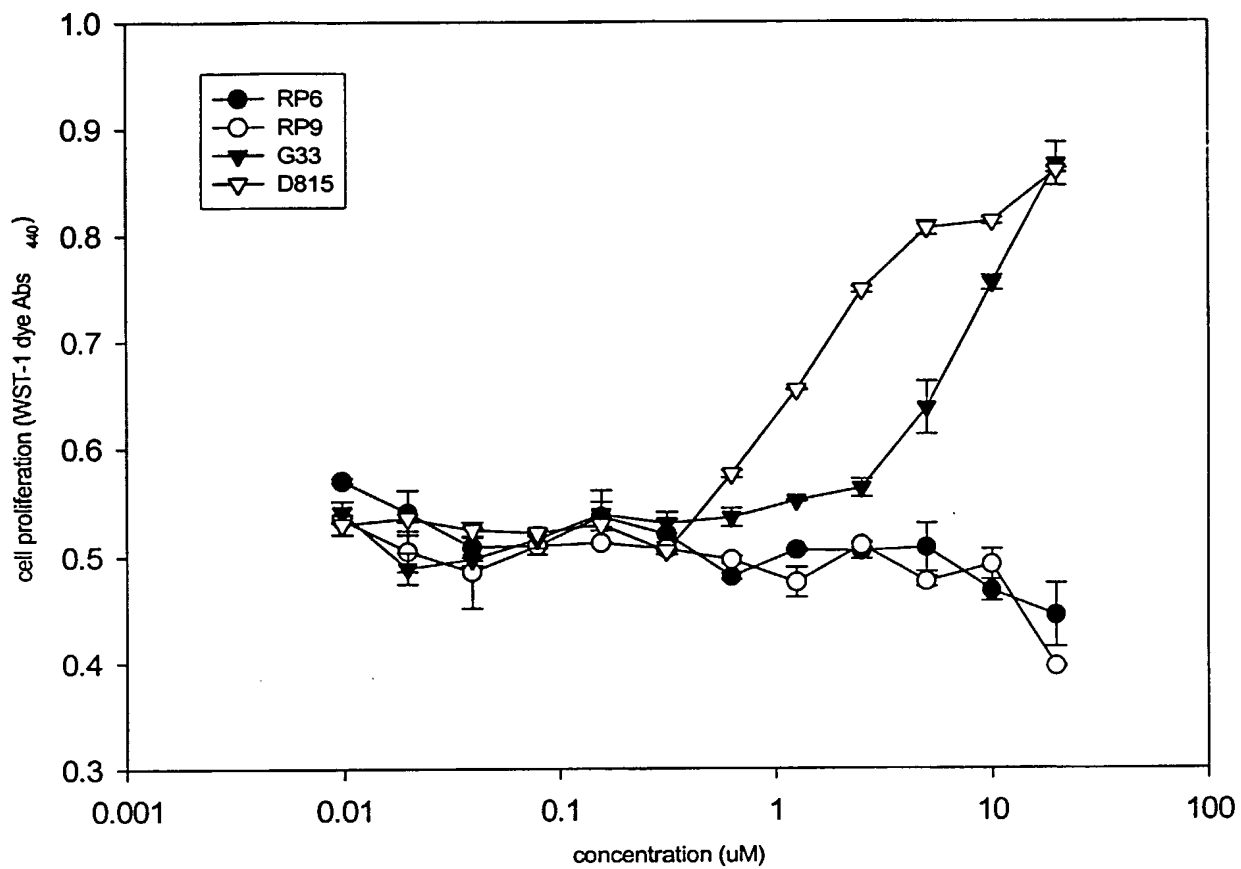
FIG. 35E

FIG. 35D

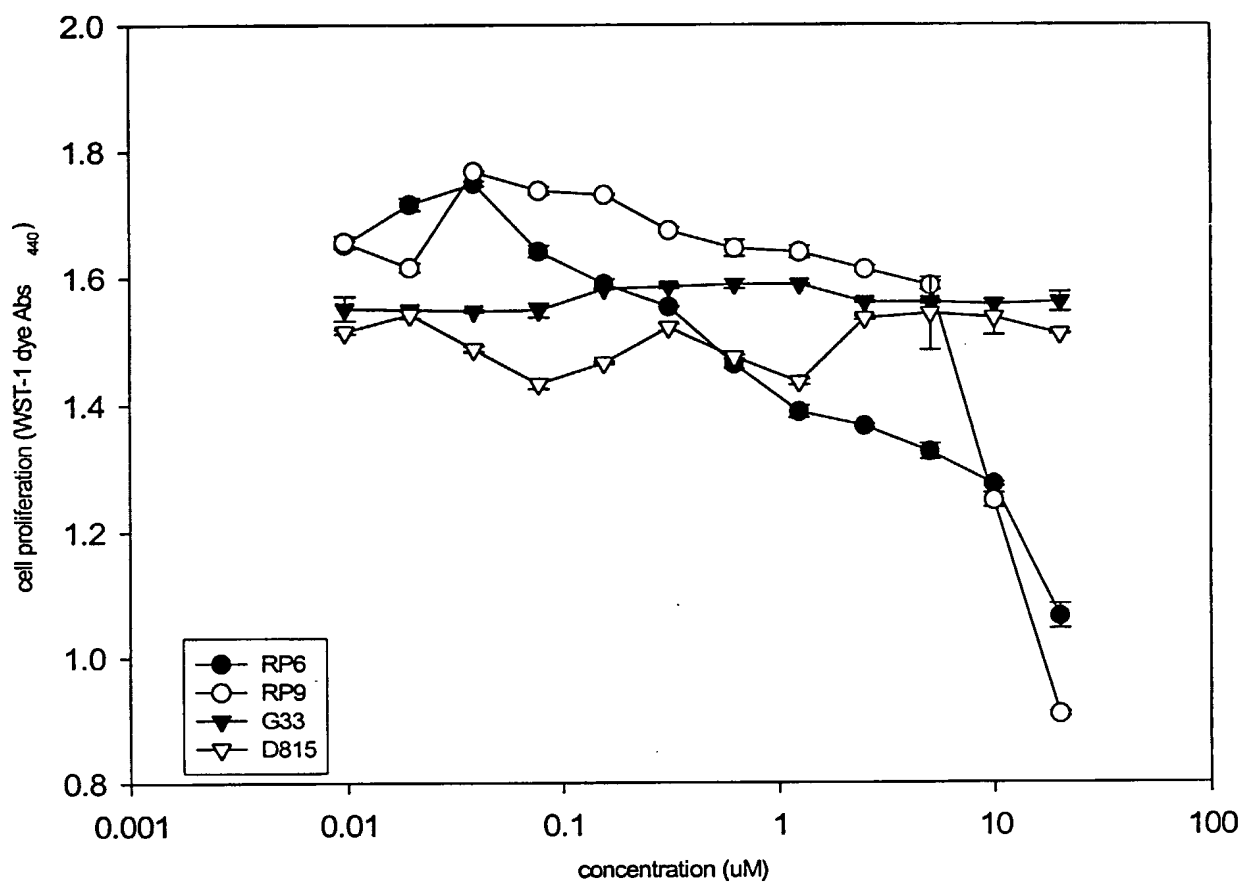
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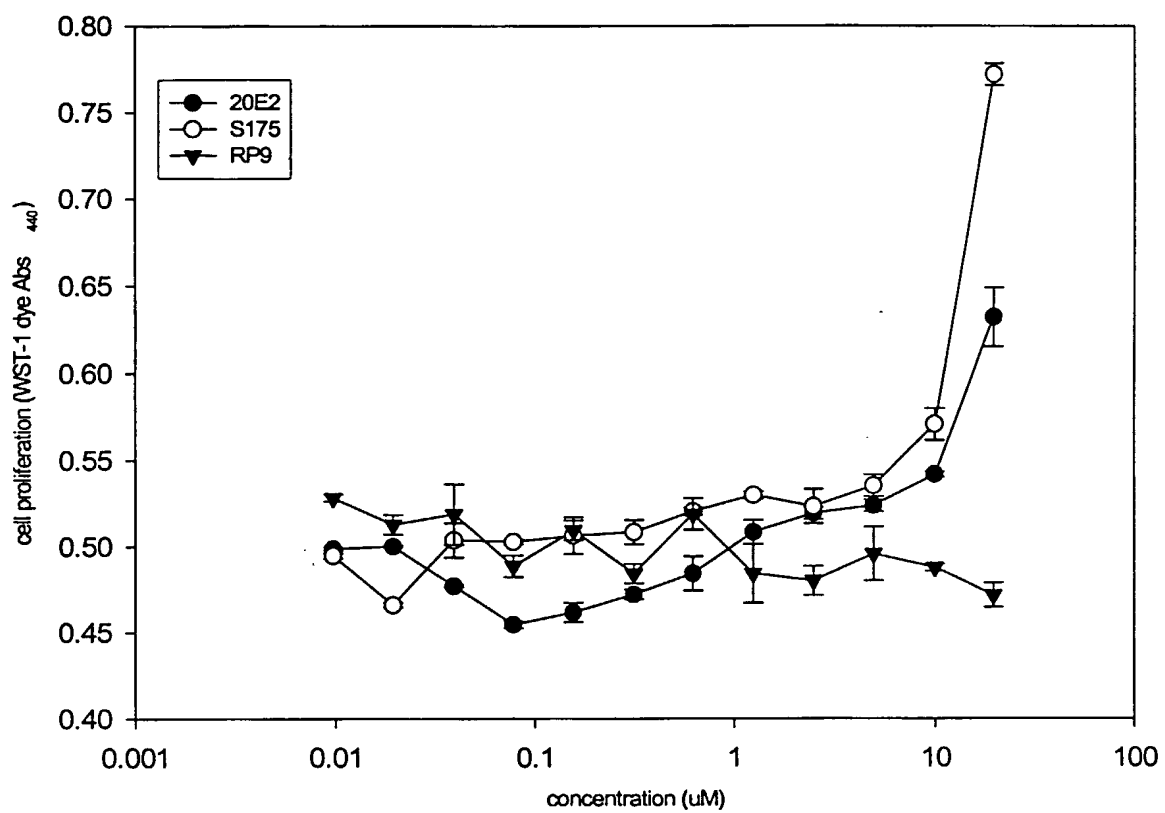
FIG. 36



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FIG. 37

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FIG. 38

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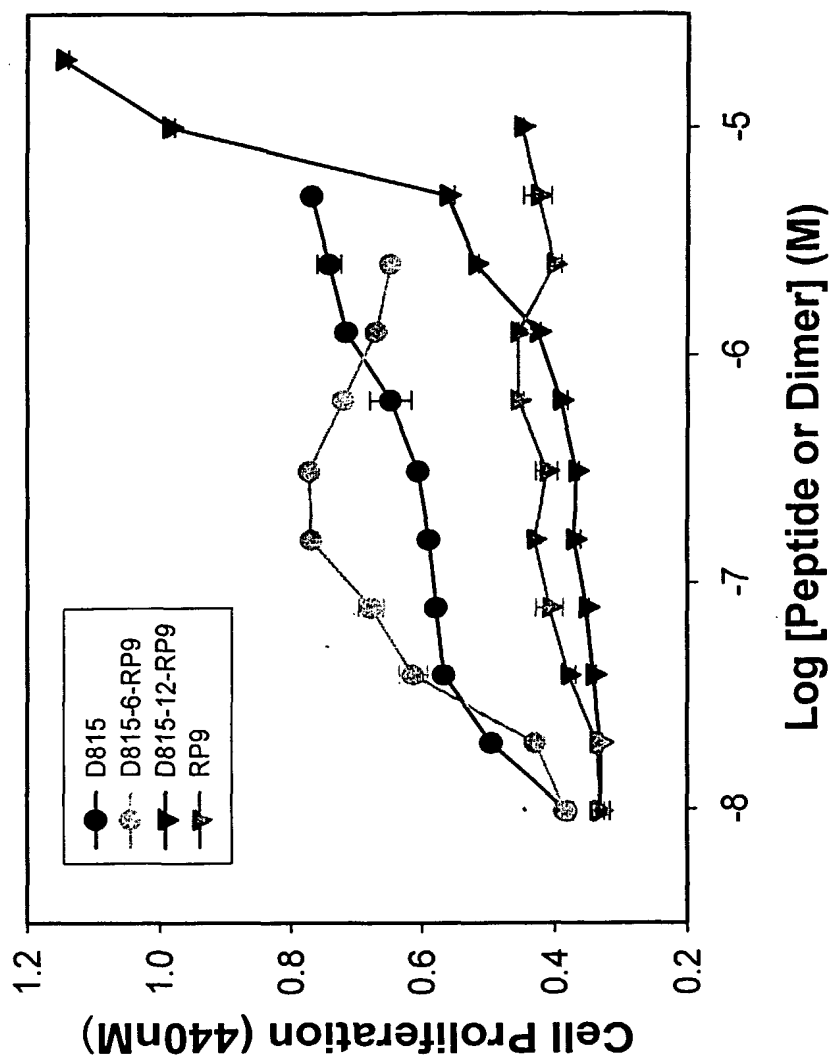


FIG. 39

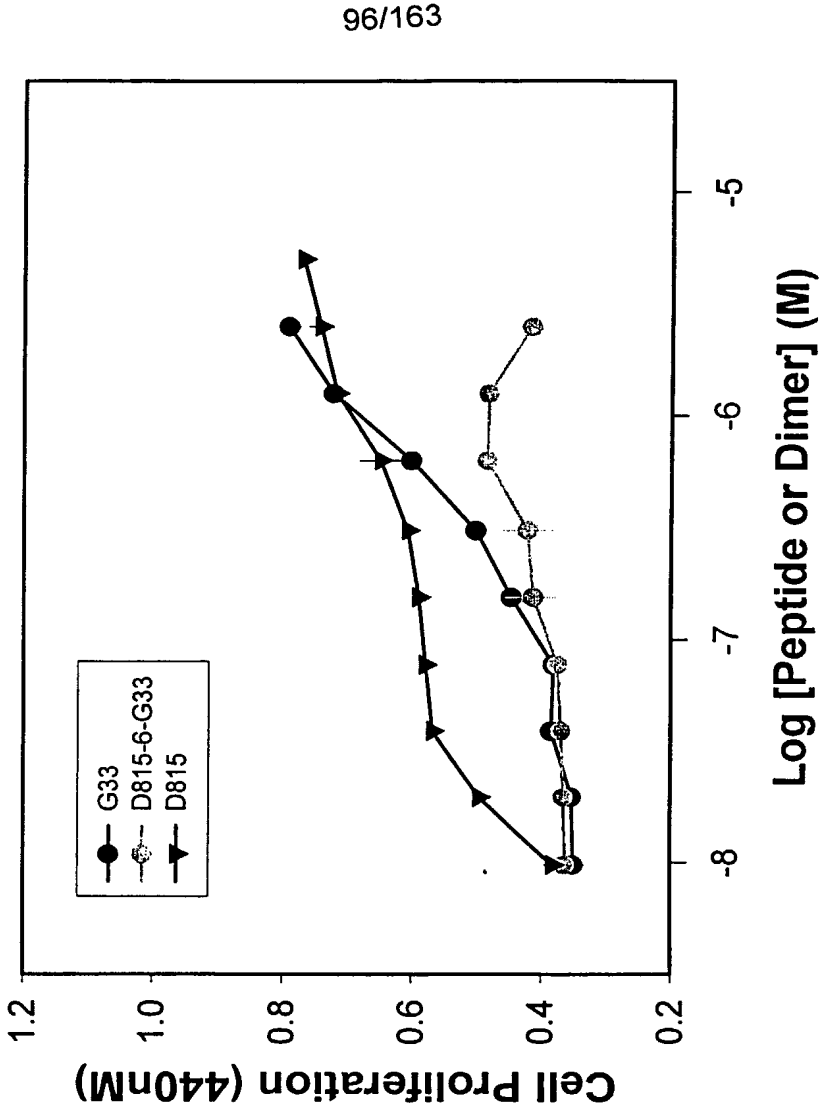


FIG. 40

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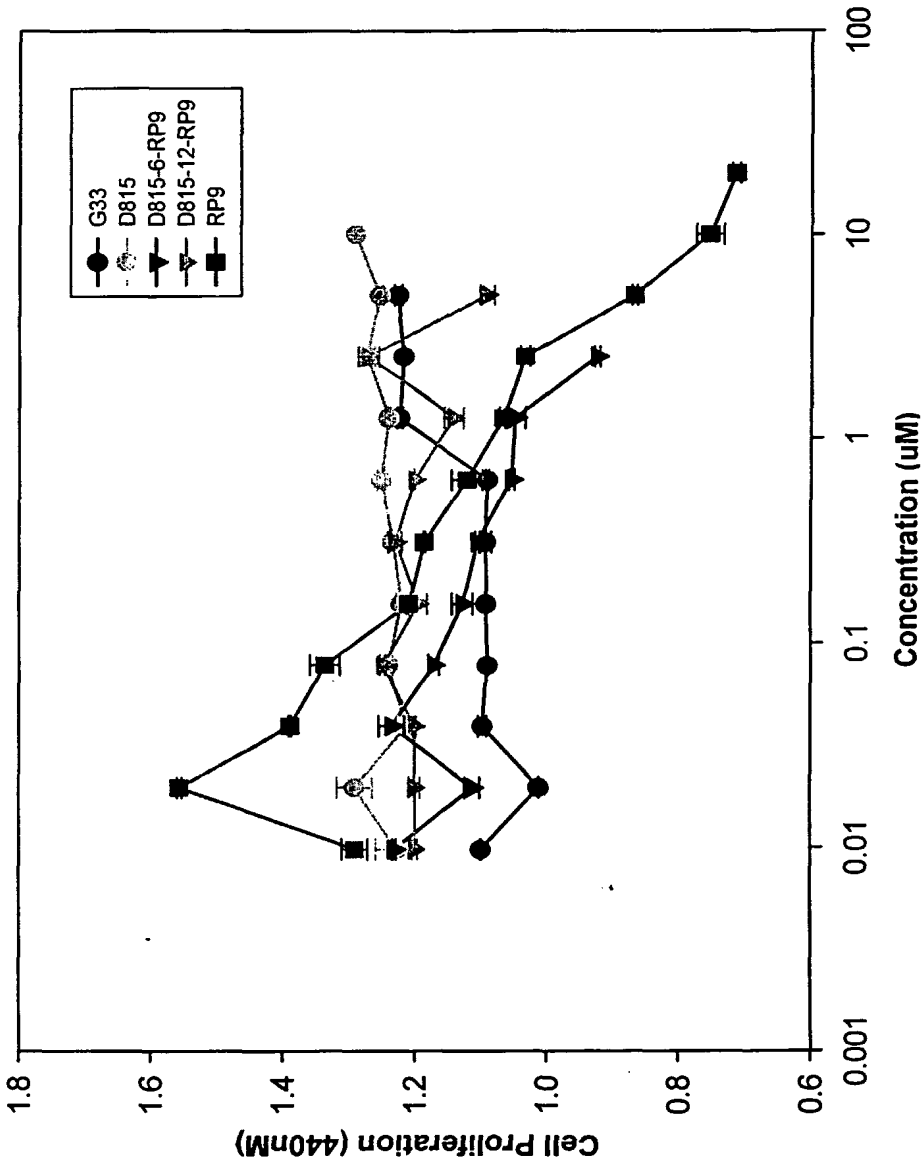
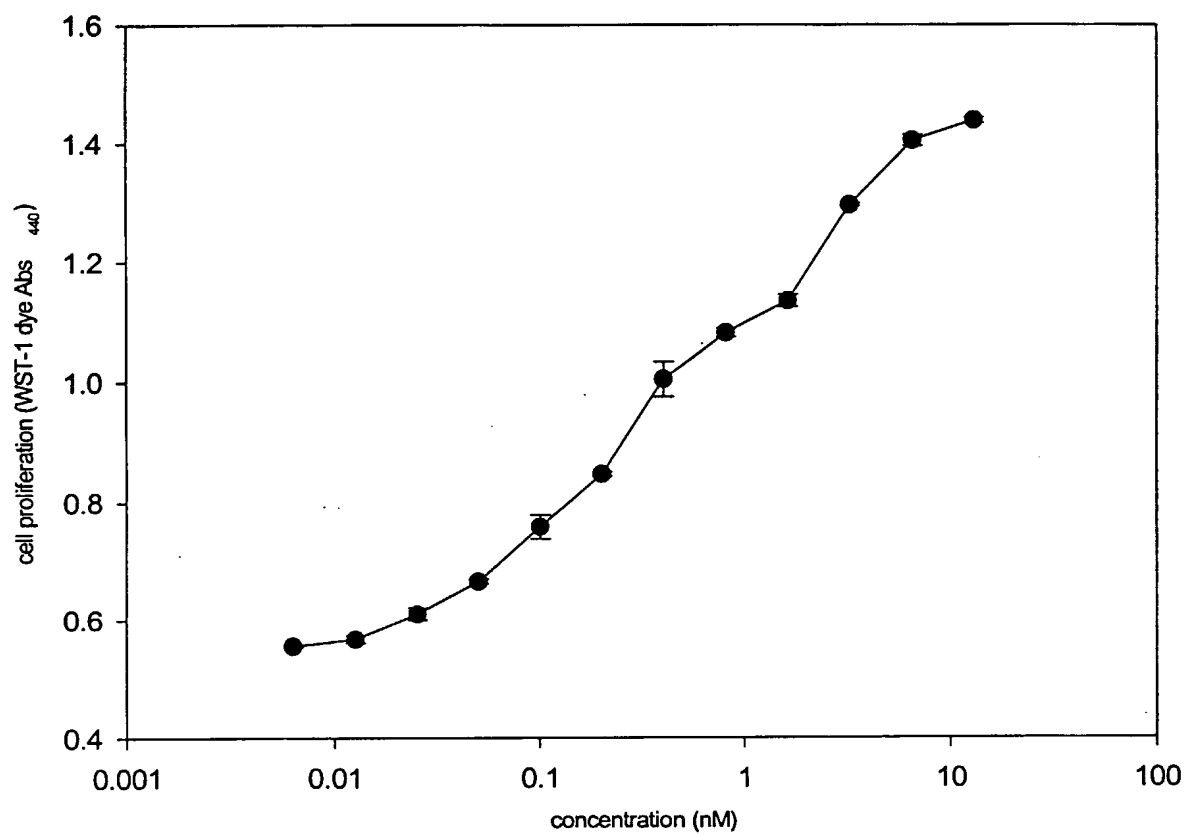


FIG. 41

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FIG. 42

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Clone #	Sequence	Etag	IGFR	LDH	IGFR/LDH	Binder
IGFR-G33-4-A1		15.5	10.3	1.1	9.5	HIT
IGFR-G33-4-A2		4.8	2.9	1.2	2.5	HIT
IGFR-G33-4-A3		14.9	16.1	1.5	10.6	HIT
IGFR-G33-4-A4	PAMAVGYPPCAKSTYERGRGSALESRCYQAAAGAP	8.6	5.4	1.3	4.3	HIT
IGFR-G33-4-A5		23.5	12.1	3.4	3.6	HIT
IGFR-G33-4-A6		10.8	5.2	1.2	4.4	HIT
IGFR-G33-4-A7	MC	13.3	5.0	0.8	6.2	HIT
IGFR-G33-4-A8	PAMACKVC*CCSVSCYDGFPRSGAHPGRRWAAAGAP	6.0	1.7	1.0	1.8	CAND
IGFR-G33-4-A9	PAMAFKVSLSGESFYEWFAGLVRDPTCGWTAAGAP	10.8	6.3	1.1	5.7	HIT
IGFR-G33-4-A10	MC	6.7	8.9	2.1	4.2	HIT
IGFR-G33-4-A11	AGHGACEFQVMFG*LVHLLGFPGRGLGKGLAAGA	5.8	5.8	1.2	4.9	HIT
IGFR-G33-4-A12	RPWRGSLRLVGRRVECYCAERGATRGW*CAAAAGAP	3.3	2.6	1.1	2.4	HIT
IGFR-G33-4-B1	AGHGDGALSCKAAVVAWVPVQTAGLRVRVAAAGAP	8.1	3.2	1.2	2.6	HIT
IGFR-G33-4-B2	PAMAPRLYQGCPESEFYANTAGHVSALYGWAAAGAP	4.8	4.1	1.1	3.8	HIT
IGFR-G33-4-B3	PGHGVSVRAGVSGMLRREVAG#CVSAWEGLCGRCA	6.4	2.0	0.9	2.2	CAND
IGFR-G33-4-B4	PAMAGMDPQ#CTVASRRWFASPV#VVWRC#AAAGAP	5.8	5.4	2.6	2.1	HIT
IGFR-G33-4-B5	PAMAGMFSQTCPEGYGWFAGQASDSSLCAAAGAP	15.8	2.5	0.9	3.0	HIT
IGFR-G33-4-B6	PAMAPLGRSCAGAY*VGCGRVAF#RCWAAAGAP	7.7	2.3	1.2	1.9	HIT
IGFR-G33-4-B7	PAMAGILCPSCPHFLVDS#AAQDAAGQWPSAAAGAP	7.2	3.3	1.2	2.8	HIT
IGFR-G33-4-B8	MC	4.8	1.4	1.0	1.5	
IGFR-G33-4-B9	PAMARRIPRECGDSFYVGLRWLVENPRSDWAAAGAP	6.2	1.9	1.0	1.9	CAND
IGFR-G33-4-B10	PAMADRIQVQCPDSFYGWFAVQEPGTSGLAAAGAP	8.5	3.5	1.0	3.5	HIT
IGFR-G33-4-B11	PAMAGLPS*SCRVAMYKGQAAWSCSAAAGAP	4.9	3.9	0.9	4.3	HIT
IGFR-G33-4-B12	RPWRLILVTLVREASMTGSGVMYPRRGAGPAEGA	3.6	2.6	1.0	2.5	HIT
IGFR-G33-4-C1		27.7	24.5	1.0	24.5	HIT
IGFR-G33-4-C2	PAMAGSARQVCVDGVVGVWREG*VVDQWL#RAAAGAP	28.2	8.4	1.9	4.5	HIT
IGFR-G33-4-C3	PAMAGIMQACEGGFTDCLWSLISGASSGRAAAGAP	29.7	5.3	1.3	4.0	HIT
IGFR-G33-4-C4	RPWRVSSLRHVRVTCGELFGGVSELFCLCAAAGAP	7.5	5.6	1.1	5.0	HIT
IGFR-G33-4-C5		4.5	4.4	1.2	3.8	HIT
IGFR-G33-4-C6	PAMAGLIYMSCLAYFDDLIERRLEKPG#RFAAAGAP	36.1	22.9	6.3	3.7	HIT
IGFR-G33-4-C7	PAMAGIMPQCGETSGKCMRGQVSLRWWSAAAGAP	10.0	1.7	1.1	1.6	CAND
IGFR-G33-4-C8	PAMAFILPRSCEDLYDFLASKVHVFRSLAAAGAP	9.7	6.9	1.9	3.6	HIT
IGFR-G33-4-C9	PAMACMSSQPCGESFYDFWFAQVRDPGWESAAAGA	23.3	19.4	9.5	2.1	HIT
IGFR-G33-4-C10	RPWRGWAIRGVHRRC*GAWRGQVAQELCR#AAAGA	30.2	9.3	4.3	2.2	HIT

FIG. 43A-1

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Clone #	Sequence	Etag	IGFR	LDH	IGFR/LDH	Binder
IGFR-G33-4-C11	PAMAGIASHTCPGGFYEFWFAQSRAPGWDGAAAGAP	10.6	6.7	1.1	6.1	HIT
IGFR-G33-4-C12		19.2	30.2	5.2	5.9	HIT
IGFR-G33-4-D1	PAMAGRIARACDPSMFWLAGQSGSQSGWQAAAGAP	2.6	1.8	1.1	1.7	CAND
IGFR-G33-4-D2	PAMARPI SPLC#RRSKDEEDASRVSLPGFFCAAAGAP	6.2	5.1	1.2	4.4	HIT
IGFR-G33-4-D3	MC	31.0	8.5	1.0	8.3	HIT
IGFR-G33-4-D4	PAMADYKDDDDKTFYA CLASLMAGTPRQYRTPWARCPAAAGAP	4.8	1.7	1.1	1.5	CAND
IGFR-G33-4-D5	MC	19.5	2.2	1.0	2.2	HIT
IGFR-G33-4-D6	RPWRVNTSESCL#FVCSLFSGYECWVG*WAAAGAP	3.4	1.1	1.0	1.1	
IGFR-G33-4-D7	PAMAGMGVQSCHDSFYGFWGCLFSDAEGDRAAGAP	20.7	15.2	7.0	2.2	HIT
IGFR-G33-4-D8	PAMAGDTSRACPESLNG.FCVVGVALRRWIAAGAP	20.1	7.0	1.0	7.1	HIT
IGFR-G33-4-D9		14.5	6.6	1.7	3.8	HIT
IGFR-G33-4-D10	PAMARWWRGLCGERWYHRGWVQVQFPWERGAAAGAP	6.4	1.1	1.1	1.0	
IGFR-G33-4-D11	RPWRVPWVLEMPYGNANLVFYDALQRLAAAGAP	27.7	19.7	1.2	16.5	HIT
IGFR-G33-4-D12	AGHGVCLYLAGVGEALGGGRVSGFAIGQVRAAGAP	29.8	16.9	3.3	5.0	HIT
IGFR-G33-4-E1	PAMAGRIKEFCRSFYDQVACLKVGPSWGGAAAGAP	11.0	13.2	2.0	6.5	HIT
IGFR-G33-4-E2	PAMAGRIKEFCRSFYDQVACLKVGPSWGGAAAGAP	18.9	16.0	3.7	4.3	HIT
IGFR-G33-4-E3	PAMAGISSRSCAENLRFGRWQSGDVWDCLAAAGAP	22.4	21.3	0.9	22.9	HIT
IGFR-G33-4-E4	PAMASRIPQWCRDSFYEFWFECLLGPRESRAAGAP	14.5	7.3	1.2	6.1	HIT
IGFR-G33-4-E5	PAMAGAESCYRAKSFYDGLGCLVGEAWWGGAAAGAP	7.8	14.3	1.9	7.4	HIT
IGFR-G33-4-E6	PAMARSGAPRCHDPFYEFWFAVEAQEPLRCEAAAGAP	6.0	3.1	1.0	3.1	HIT
IGFR-G33-4-E7	PAMAGMGVQSCHDSFYGFWGCLFSDAEGDRAAGAP	13.9	13.9	1.9	7.4	HIT
IGFR-G33-4-E8	PAMADISFESCLAQLLWGRAGEGSKRLWRCAAGAP	11.9	17.1	3.5	4.9	HIT
IGFR-G33-4-E9	PAMANTFLYPCRDPPFYHSLADLVGVAMQCAGAAAGAP	23.2	24.5	5.2	4.7	HIT
IGFR-G33-4-E10	PAMARRIPRECGDSFYAGRLCLVESPRSDWAAAGAP	9.4	5.8	1.7	3.3	HIT
IGFR-G33-4-E11	PAMASIVCFCEDSFYNNWFAAQVADTRGLWAAAGAP	24.1	33.5	10.1	3.3	HIT
IGFR-G33-4-E12		1.2	0.9	1.0	0.9	
IGFR-G33-4-F1	PAMAWSHSHAYTESYYDWFAAQVLSAGSGRAAGAP	0.9	1.1	0.9	1.3	HIT
IGFR-G33-4-F2	PAMAGRIKEFCRSFYDQVACLKVGPSWGGAAAGAP	7.2	8.5	0.9	9.7	
IGFR-G33-4-F3	PAMARSRPACGDSFYGWFECEVSGLRRGAAAGAP	2.2	1.4	1.0	1.4	
IGFR-G33-4-F4	PAMAGISYPACEESFYDCLASLVLSPWGSGAAAGAP	12.1	5.2	0.8	6.7	HIT
IGFR-G33-4-F5	PAMAGRIKEFCRSFYDQVACLKVGPSWGGAAAGAP	16.7	24.2	7.3	3.3	HIT
IGFR-G33-4-F6	PAMAVVAGQYCRDSFYDRLSALVGDWRCGAAAGAP	13.6	7.4	1.9	3.8	HIT
IGFR-G33-4-F7	PAMACTASRFCAVSFYEFWFAAQPLDLGGDSAAAGAP	12.5	16.9	1.2	13.8	HIT
IGFR-G33-4-F8	PAMAGITLQSCGGGFYELLASVVGDTCRLAAAGAP	20.2	10.9	1.0	11.3	HIT
IGFR-G33-4-F9	PAMAGYICRSCQGSFYGC LAALVRDPRCSRRAAGAP	24.7	33.0	8.8	3.7	HIT
IGFR-G33-4-F10	PAMAGRIKEFCRSFYDQVACLKVGPSWGGAAAGAP	7.1	10.6	1.2	9.1	HIT
IGFR-G33-4-F11	RPWRVAGAPRCHDPFYEFWFAVEAQEPLRCEAAAGAP	1.0	1.0	0.8	1.2	

FIG. 43A-2

Clone #	Sequence	Etag	IGFR	LDH	IGFR/LDH	Binder
IGFR-G33-4-F12	PAMAGMGVQSCHDSFYGWFGLFSDAEGDRAAAGAP	7.6	4.7	0.6	8.0	HIT
IGFR-G33-4-G1	PAMASICGQSCRDPFYAGLRGLLLEPLQGAAGAP	17.6	18.5	1.0	19.5	HIT
IGFR-G33-4-G2	PAMAGVMSKCCSGSFYDWLADLVPEASWAAAGAP	6.5	5.7	1.0	5.5	HIT
IGFR-G33-4-G3	PAMASFGEACGGSFYDCLAGLMRDSSVRAAAGAP	18.4	7.9	1.1	7.4	HIT
IGFR-G33-4-G4	PAMASFYTCMETLLDGFGGQAFNRCRRTAAAGAP	22.5	20.1	1.3	15.6	HIT
IGFR-G33-4-G5	PAMARVIYPTCPRDFYGGLAALVFGPHVCGAAAGAP	22.8	21.7	1.9	11.5	HIT
IGFR-G33-4-G6	PAMAGIGSQACTDPFYWFEGLVNSNGWCRAAAGAP	5.9	5.3	1.2	4.3	HIT
IGFR-G33-4-G7	PAMAGRIKEFCRSFYDQVACLKVGPSWGGAAAGAP	18.8	2.1	1.0	2.1	HIT
IGFR-G33-4-G8	PAMAGAESCYRAKSFYDGLGCLVGEAWGGAAAGAP	23.6	30.3	3.7	8.2	HIT
IGFR-G33-4-G9	PAMADMMSQVCSQSMGTGRFSVDYDGLRCLAAAGAP	17.3	4.6	0.9	5.1	HIT
IGFR-G33-4-G10	PAMARRIPRECGDSFYAGLRCLVESPRSDWAAAGAP	26.8	24.6	5.4	4.6	HIT
IGFR-G33-4-G11	PAMARVIOEACGGSFYDGLACLVPQGWGAAAGAP	3.3	1.5	0.9	1.7	CAND
IGFR-G33-4-G12	PAMAGGRSVACQESFYALLGCVVMGPGGSAAGAP	24.1	32.1	12.1	2.7	HIT
IGFR-G33-4-H1	PAMAGISFRSCLQALIASAGNASSEMGRSAAAGAP	5.9	5.8	1.2	4.8	HIT
IGFR-G33-4-H2	PAMAGIRDSYCQAFYDWFAGLVDDGLFCQAAAGAP	9.2	4.4	1.0	4.4	HIT
IGFR-G33-4-H3	PAMAGISYQSCEDSFYAFWFACTVLDTRGGAAAGAP	17.8	16.0	1.8	8.9	HIT
IGFR-G33-4-H4	PAMARVIYEACGGSFYDGLACLVPQGWGAAAGAP	3.1	3.2	1.1	2.8	HIT
IGFR-G33-4-H5	PAMADMPLLECLDPPFYSWFAGQVSDPRFCGAAAGAP	20.1	7.5	0.9	8.0	HIT
IGFR-G33-4-H6	PAMARVIOEACGGSFYDGLACLVPQGWGAAAGAP	5.1	2.4	0.8	2.9	HIT
IGFR-G33-4-H7	PAMAGRIKEFCRSFYDQVACLKVGPSWGGAAAGAP	12.9	11.1	1.1	9.8	HIT
IGFR-G33-4-H8	MC	23.4	23.5	1.6	14.7	HIT
IGFR-G33-4-H9	PAMAHISFHSCLEALQDPEWGQPSAAWRNCAAGAP	1.2	1.1	0.8	1.3	HIT
IGFR-G33-4-H10	PAMAMTAQESCPDSFYECCLAVLVGDRWGGWAAAGAP	7.9	10.4	2.8	3.7	HIT
IGFR-G33-4-H11	PAMAHISFHSCLEALQDPEWGQPSAAWRNCAAGAP	16.8	23.7	1.3	18.1	HIT
IGFR-G33-4-H12	PAMAGTISQCCEENFYAGLAHLAHLAGVQWGGAAAGAP	20.4	19.0	4.7	4.0	HIT

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FIG. 43A-3

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Clone #	E-Tag	IGFR	IR	Sp/Irr	Binders
B10	2.6	29.0	1.0	29.0	HIT
D1	17.0	26.8	1.1	25.1	HIT
A4	25.2	25.6	1.0	25.6	HIT
A6	22.3	23.3	1.1	21.2	HIT
B1	18.0	22.8	0.9	25.3	HIT
E4	20.8	20.6	1.1	19.1	HIT
C11	22.7	20.4	1.3	16.2	HIT
C2	19.9	19.1	1.0	19.1	HIT
B6	22.6	19.0	1.2	15.3	HIT
A12	13.9	18.8	1.0	18.4	HIT
D2	22.5	17.2	1.1	16.1	HIT
C1	21.6	16.9	1.1	15.2	HIT
F10	11.4	16.6	0.9	18.2	HIT
B11	7.8	16.1	0.8	20.8	HIT
A9	16.3	15.9	1.3	11.8	HIT
A10	8.3	15.6	1.0	16.2	HIT
B9	5.9	14.3	0.9	15.4	HIT
A3	14.1	13.3	0.9	15.1	HIT
F2	9.6	13.3	0.9	14.8	HIT
A7	10.9	13.2	1.1	12.5	HIT
G3	9.6	13.2	1.0	13.8	HIT
A11	4.7	12.7	0.8	15.0	HIT
B7	19.7	12.5	0.9	13.5	HIT
D5	21.8	11.8	1.3	9.3	HIT
E8	11.8	11.8	1.3	9.0	HIT
D10	15.1	11.6	1.0	11.6	HIT
D8	11.1	9.2	0.9	10.6	HIT
D12	5.4	7.4	0.8	9.0	HIT
A5	16.0	7.3	1.3	5.5	HIT
F6	4.2	6.9	1.0	7.1	HIT
G5	4.8	6.4	1.3	5.1	HIT
C6	13.6	5.9	1.1	5.4	HIT

FIG. 43B-1

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Clone #	E-Tag	IGFR	IR	Sp/Irr	Binders
D3	TDDKTFYFCLASLLAGTQQQSRGAWERCG	5.1	5.9	0.9	HIT
A2	DDDKAFYSCLASVLTGSPHPGRSRWERCR	18.6	5.5	0.9	HIT
B2	DDDKTFYSCLESMLTGTTPPCRHHGERGR	8.0	5.5	1.0	HIT
D7	DDDKTFESCLEALVSGRRERGLWYRCR	10.6	5.5	1.1	HIT
C12	DDDKAFYSCLSSLLAGTRERHRTWPRCG	12.0	5.3	1.0	HIT
B5	DDDKTFHSCLAALVTGTPQKRGPWERC	20.0	5.1	1.3	HIT
E2	DDDKTFYCLASLQTVTRLGDRVPWERC	18.0	4.6	0.7	HIT
F3	DDDKTFYCLVSLNLTAKPNRGQWEGCR	3.7	4.5	3.2	HIT
H2	DDDKSFYSCLASLSNCTPGLLRQWERC	7.2	4.4	5.4	HIT
B4	DDDKTFYSCLSLLASTPQPNRGAWLCRR	11.9	4.3	3.6	HIT
G12	DDDKSFYSCLASLSNCTPGLLRQWERC	2.2	4.2	4.4	HIT
F5	MC	8.4	4.0	4.3	HIT
A1	DDDKTFYSCIGALLSGAPQTYRGPAGCR	8.1	3.9	3.9	HIT
E11	DDDKTFYSCLVSLNLTAKPNRGQWEGCR	4.9	3.8	3.9	HIT
F9	DDDKHFYSCLSLLTAPPQSTRGPAGRHC	3.9	3.7	3.6	HIT
E3	DDDKTFYSCLASLLNGTQPNGGQWVRCR	1.7	3.6	4.5	HIT
G4	DDDKVFYTCLASLSTGTPQRSGEWQRCR	9.4	3.3	2.5	HIT
E5	DDDKTFYSCLVSLNLTAKPNRGQWEGCR	4.6	3.2	3.7	HIT
C10	DDDKPFYSCLASLIQGTPLPERGMWERC	6.3	3.0	3.0	HIT
C7	MC	10.6	2.9	3.0	HIT
H5	DDDKTFYSCVSWLLTGARQDGVWERC	5.5	2.9	2.2	HIT
H3	DDDKTFYSCLVSLNLTAKPNRGQWEGCR	4.6	2.8	2.5	HIT
C3	DDDKAFYGCALALLTGARQPSRGVGERCF	6.0	2.7	1.8	HIT
F1	DDDKTFYSCLVSLNLTAKPNRGQWEGCR	1.5	2.6	3.4	HIT
B3	DDDKTFYSCLASLLAGSPQKRGWEYCR	8.6	2.5	2.5	HIT
D11	DDDKPFYSCLESVLTGTPQADRGVWERC	4.9	2.4	2.7	HIT
E10	DDDKTFYSCVLSLRSAGLSGRWERC	5.0	2.3	2.7	HIT
F12	DDDKTFYFCLATLLTGPPVPPNREPWACYR	2.5	2.2	2.7	HIT
D4	DDDKIFYSCVSLNLTGTPPPVPPNREPWACYR	3.0	2.1	1.9	HIT
E6	DDDKTFYSCVSLNLTGTPPPVPPNREPWACYR	2.7	2.1	1.5	HIT
F11	DDDKSFYSCVSLNLTGTPPPVPPNREPWACYR	3.5	2.1	1.7	HIT
A8	DDDKTFYSCVSLNLTGTPPPVPPNREPWACYR	8.3	2.0	1.9	HIT
F7	DDDKKFYSCVSLNLTGTPPPVPPNREPWACYR	2.7	2.0	2.1	HIT
F8	DDDKVFYSCVSLNLTGTPPPVPPNREPWACYR	2.0	1.8	1.7	CAND
E12	YDDKRFYSCVSLNLTGTPPPVPPNREPWACYR	1.0	1.7	1.7	CAND
H1	DDDKTFYSCVSLNLTGTPPPVPPNREPWACYR	1.9	1.7	1.9	CAND
H8	DDDKTFYTCVSLNLTGTPPPVPPNREPWACYR	1.2	1.7	1.4	CAND

FIG. 43B-2

Clone #	E-Tag	IGFR	IR	Sp/Irr	Binders
G9	1.5	1.6	1.1	1.5	CAND
D6	1.3	1.5	1.1	1.5	CAND
H6	3.2	1.5	1.3	1.1	
E7	3.2	1.4	1.1	1.3	
F4	1.4	1.4	1.2	1.1	
G6	1.4	1.4	1.0	1.5	
G11	1.6	1.4	1.0	1.4	
H4	4.7	1.4	1.0	1.4	
B8	9.1	1.3	1.0	1.3	
C8	7.7	1.3	1.4	1.0	
E1	2.3	1.3	0.9	1.4	
G10	1.2	1.3	1.2	1.2	
H10	3.5	1.3	1.0	1.3	
H11	2.6	1.3	1.0	1.3	
D9	1.1	1.2	0.9	1.3	
E9	1.1	1.2	1.1	1.1	
C9	1	1.1	1.1	1.0	
G1	0.9	1.1	0.9	1.3	
H7	2.2	1.1	0.9	1.1	
H9	3.1	1.1	1.1	1.0	
C5	3.3	1.0	1.0	0.9	
G7	0.9	1.0	0.9	1.1	
H12	3.7	1.0	1.0	1.1	
C4	1.1	0.9	0.9	1.0	
G8	0.7	0.9	1.0	0.9	
G2	1.1	0.8	1.0	0.8	
B12	9.3	13.2	0.7	18.3	HIT

FIG. 43B-3

IR

Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
B5	FHENFYDWFARKDSGGSGGSDLCVLEELFWGDSLFDYCTG	17.0	16.9	0.5	35.8	0.0
A3	FHENF.DWVVRQVSGSGGSGNLCVLEELFWGASLFGECSG	13.0	11.8	0.3	35.8	0.0
A8	SHGNFSEWVRQYGGSGGSDLCVLEELYWGASLFGYCSG	13.2	13.1	0.4	33.2	0.0
C7	FQESFYDWFVR.VTGGSGGSDLCGVEDLVWGSALS GYCAG	15.1	14.7	0.5	30.6	0.0
B4	FHENFNDWFVREVSGSGGSDLCVLEELFWGASLFSYCSG	13.2	11.7	0.4	27.6	0.0
B11	SHENFYDWFVR.GPGSGGSGSHLCVLEELFWGDSLFGACPG	10.9	9.1	0.3	27.0	0.0
A9	FHENFYDWFARQVSGSGGSHLCVLEELFWGASLFA.CSD	10.7	12.3	0.5	25.7	0.0
A6	FPDNFYDWFVR.VSGSGGSGSHLCVLEELFWGASLFGYCSG	11.6	8.7	0.4	19.8	0.1
A4	FQENFYDWFGRQISGGSGGSGPLCDVEELFWGVSLFGYCTG	13.6	12.1	2.6	4.6	0.2
C8	FQENFYDWFVR.ASGSGGSGSHLCALVEEQFWGSSQFRYCSG	16.0	14.5	3.2	4.5	0.2
A10	FHENFYDWFARQVYGGSGGSHLCVLEELFWGASLFGYCSG	10.6	6.0	1.5	3.9	0.3
D11	FHENFYDRIVRQVAGSGGSGSALCVREELF.GDSLFGDCSG	12.4	5.5	1.5	3.6	0.3
D4	FHKNFYDWFDRQVSGSGGSSRLCDLEELFWGASL.GHCSG	15.4	9.8	3.9	2.5	0.4
C1	FHENFYDWFIRQDSGGSGGSHLCAFEELLGASLFGYCSG	16.8	2.7	1.3	2.1	0.5
D12		11.7	8.7	4.6	1.9	0.5
D8	SNENFYDWFDR.VSGSGGSGGSHLCLEELSWGASLFGYCYG	15.8	9.6	7.4	1.3	0.8
C11	FHESFYDWFDRQVSGSGGSHLCVLEE.ELGASVFGCCSG	11.0	5.8	5.4	1.1	0.9
C4	FHETFYDWFDR.VSGSGGSGEELFGGASLFGYPSG	16.7	13.2	15.0	0.9	1.1
D1	SHENFYDWFGRQVSGSGGSGNLCDLDEVS.GASLCGYRSG	16.2	5.5	7.1	0.8	1.3
C6	FH.NFYDWFQCQPEWIPMTLAVLTCAVLEEPIWGDLSFGYG.E	16.1	1.7	2.2	0.8	1.3
A5	SHENFYDWFVRQV.GSGSGGSHLCVLEELGGASLMGSCSG	16.0	8.7	12.9	0.7	1.5
B8	SHENFYDWFVR.VSGGAAAGAPPAMASHENFYDWFVR.VSGG	15.2	8.9	13.9	0.6	1.6
D2	FHENFYDWFIR.VGGSGGSGSDLCVLEDDCSRAAAGAP	13.9	8.4	13.1	0.6	1.6
A2	DYKDAVS GTFHDAFYEFWVR.VGS	13.4	6.9	12.6	0.6	1.8
C12	FHENFYDLVPSAGSWIRWLWRF.PVRLGRTVLGCFSDR.LFW	9.2	4.4	6.8	0.6	1.5
B9	FHENFYDWFDRQVSGSGGSGSVRAAAGAP	17.8	8.2	16.3	0.5	2.0
B1	VHENFYDWFDRQVSGSGGSQLCDL.EVIWGASLFGYCTG	18.1	7.3	13.4	0.5	1.8

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FIG. 44A-1

Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
B2	FHENFYDWFDR.VSGSGSGSGSHLCVPPEQFWGASRFGYCSA	16.2	7.0	13.6	0.5	1.9
D9	FHDNFYDWFVRQVSGSGSGSGRQCQASL.GYCSG	17.0	6.6	13.8	0.5	2.1
D5	OUT OF FRAME	16.1	4.8	10.5	0.5	2.2
C9	FHEDFYDWFVR.VPGSGSGSGSHLCVPGYCSSL	17.2	5.1	14.2	0.4	2.8
B7	FRENFYDWFVC.VSGSGSGSGSNLCVLEEAAGAP	15.9	4.0	10.6	0.4	2.7
D6	OUT OF FRAME	15.6	4.2	12.8	0.3	3.0
C5	GHDNFYDWFVRQVSGSGSGSGSHLCV.GAFPWGYCSD	15.2	3.6	10.7	0.3	3.0
D3	FH.NFYDWFVRQVYGGSGSGGTGAAAGAP	16.2	3.5	12.0	0.3	3.4
C10	BAD SEQUENCE	11.2	2.5	7.6	0.3	3.1
A7	FHENFYDWFGRQVYGGSGSGSPVCILGELS.GGALFGDCSG	15.5	1.8	5.1	0.3	2.9
A12	FHENFYDWFVR.LSGSGSGSGSHLCVPEERLWGDPLFGYCSCG	8.7	1.2	3.5	0.3	3.0
D7	FH.NFYDWFVRQVSGSGSGSGSHPAR	16.2	3.0	11.9	0.2	4.0
A11	FHENFYDWFVRQVTGGSGSGSGSHLCVLEELS.GAALPGYCSCG	11.8	1.0	4.0	0.2	4.1
D10	VQGSFYDWFVRQVSGSGSGSGSHLC.GSG	12.7	1.0	6.3	0.2	6.6
C3	FHENFYDWFVRQVSGSGSGSGSHRCDVEELH.CASG	16.8	0.6	2.5	0.2	4.2
A1	DYKGGYWGSEFYEGLM.LVQSGTSG	13.6	1.7	12.5	0.1	7.1
B6	OUT OF FRAME	12.7	1.0	8.1	0.1	8.1
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Non-Binders:						
B12	FHENFYDWFDRQVSGSGSGSGSHRVCVLEERFWGASLFG.CSG	7.8	0.5	1.6	0.3	3.3
B10	SHENFYDWFVHQVSGSGSGSGSHLCVLEERF.GPSLFGYCSCG	10.8	0.6	1.4	0.4	2.3
B3	FHANFYDWFVRQVSGSGSGSGSDLCVLQDMF.GGSGAAAGAP	16.9	0.7	1.2	0.6	1.7
C2	FQDNFYDWFVRQISGGSGSGSGSHLCVLESWF.GASLFGYCSCG	14.8	0.5	0.8	0.6	1.7

FIG. 44A-2

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IGFR

Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
H11	FHETFYD.LGRLVFGSGGGSGSHLCVPEELFWGTSLLSYCSG	9.3	0.4	4.0	0.1	11.4
F11	FHENFYDWFVRQVSGSGGGSHL.GSG	12.5	0.8	5.2	0.2	6.5
E2	FHENFYDWFVRQVSGSGGGSHRCGLEEPV.GASLVGYCAG	13.4	1.3	7.5	0.2	5.7
G7	FHANFYDWFVRQV.GSGSGSGSG	16.1	2.1	9.8	0.2	4.7
G12	FHEDFYDWFVRQVGGSGGGSHLCVREELF.GASLLGDCSG	9.4	1.2	5.5	0.2	4.6
H7	.HENFYDWFVRQLSGSGGGSDGSHLFGYGSG	7.2	0.6	2.7	0.2	4.5
G11	OUT OF FRAME	11.4	1.4	5.8	0.2	4.3
F7	FHENFYDWFDRQVGGSGGGSPVRTGRTVLGGFSVRLLLW	15.0	2.7	10.9	0.2	4.1
G1	SHDNFYDWFVR.VSGSGGGSPLCVLGNCSG	11.3	2.8	10.6	0.3	3.8
E8	FYDNFYHWFDR.VSGSGGGSHLCVLEERVCGASLFDYRS	13.5	0.9	3.4	0.3	3.7
E9	FSEHFYDWFARQVSGSGGGSHLCVLDERF.GASMVGYCSG	14.5	0.7	2.3	0.3	3.6
G2	FPENFYDWFDRQVSGSGGGASLFG.GSG	15.3	3.8	13.1	0.3	3.5
E3	FHENFYDWFDRQVSGSGGGSHQCVQEERFWGASLCGYCSG	15.9	1.9	6.7	0.3	3.5
E12	FHDSFYDWFVRQVSGSGGGSHLCGLEELF.GASRFGDCSG	10.0	2.3	6.8	0.3	2.9
E5	OUT OF FRAME	14.7	3.7	9.6	0.4	2.6
F8	FHGDIFYDWFVR.VSGSGGGSHLCVLEELYCSG	13.7	3.6	9.5	0.4	2.6
E6	FHDNFYDWFVR.VSGSGGGSHLCVVEERFWGGSPIGYCSG	13.3	3.0	7.3	0.4	2.5
G8	OUT OF FRAME	13.9	4.5	10.5	0.4	2.4
E1	FQDNFYDWFVRQVSGSGGGSHRCVLEGCSCG	13.4	5.8	13.3	0.4	2.3
H12	FHENFYDWFDRQVSGGSA C LFYCYCSG	9.8	3.9	8.5	0.5	2.2
F2	YHENFYDWFVR.VSGSG	14.4	6.2	12.8	0.5	2.1
H6	VHESFYDWFVR.VAGSGGGSHLCVDVDCSG	11.5	4.8	9.6	0.5	2.0
H4	FHDNFYDWFDRQVSGSGGGSPFG.RSD	11.2	5.3	10.0	0.5	1.9
H5	FH.HFYDWFDRQVSGSGGGSLLCVGEEPFWGASLFAYCSG	11.8	4.4	8.5	0.5	1.9
E7	FHENFYDWFVRQVSGSGSGG	15.4	7.8	14.0	0.6	1.8
F5	FHESFYDWFVR.VPGSGGGGSQLCVQEELFEGD S LLGDCSG	16.8	7.3	12.9	0.6	1.8
F10		13.9	5.9	10.8	0.5	1.8
E10	FHENFYEWFD R QVSGSGGGVLDERF.GACPSGYCSG	10.6	5.1	8.9	0.6	1.8

FIG. 44B-1

Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
F4	FHDNFYDWFVR.VAGSGSGSGSHLCVPEELFWGASLFGYC <u>CSG</u>	15.7	3.1	5.6	0.6	1.8
H2	FGEDFYDWFVR.VSGSGSGSGSHLCVLDLFWDASPF <u>GC</u> PG	11.4	2.5	4.6	0.6	1.8
E11	.HDNFYGFDRQVSGSGSGSGSHLCVLDLFWGASLFGYC <u>CS</u>	11.7	1.3	2.2	0.6	1.7
F12	FQENFYDWFVR.VSGDELGGASQCGSC <u>SG</u>	10.6	7.0	9.6	0.7	1.4
F9	SHESFYDWFVRQVSGSGSGSGDLCVWEELCGGAPLVG.GSS	16.0	9.9	13.3	0.7	1.3
E4	FPENFYDWFDRQVSGSGSGSGSSG	16.4	13.4	15.8	0.8	1.2
H10	FRENFYDWFERQVSGSGSGSGSHLCVLEELSWGASTFGSC <u>CSG</u>	10.8	7.8	9.1	0.9	1.2
F3	IHVDFYDWFAR.VSGSGSGSGSLCVLDLFWDASLFGD <u>CAG</u>	14.2	3.9	4.6	0.8	1.2
G6	FHASFYDWFDRQVSGSGSGSGSHLCVLEGLFWGAAPFGYC <u>CSG</u>	16.2	11.0	12.1	0.9	1.1
H3	SDANFYDWFRL.VSGSGSGSGSHLCVLEEQFWDASLFGD <u>CSG</u>	13.1	9.8	11.1	0.9	1.1
G5	FHDKFYDWFVS.VAGSGSGSGSHLCVLEDRFWGSSLSGYC <u>CSG</u>	14.7	7.1	7.9	0.9	1.1
H9	FHDNFYDWFVRQVTDGSGSGSGQLCVVEDLFWDASRFGYC.G	13.1	8.2	8.0	1.0	1.0
G3	VSEDFYEWFVR.ASGSGSGSGSNLCVLEELFWGSSLI <u>GD</u> CSG	13.7	11.7	2.5	4.6	0.2
G4	FPENFYDWFVRQVSGSGSGSGSHLCVLEEL.WGASMFGYC <u>CSG</u>	10.0	4.3	0.7	6.0	0.2
F6	FQENFYDWFVRQVSGSGSGSGSHLCVLEALFWGASLFG. <u>CSG</u>	5.6	9.0	0.4	21.2	0.0
Non-Binders:						
H1	DYKDGRRGRF.GRSSVVLWKRL.R	1.2	0.7	0.5	1.5	0.7
G10	DTKTFIGITGVLPRLSAV.GFWGGSW	1.7	0.3	0.3	0.8	1.2
G9	CHENFYVWFVSQVAGSGSGSGSRLCIM.ELFRGASLFGYSSG	2.0	0.4	0.5	0.9	1.1
F1	FHANFYDWFVR.VSGSGSGSGSHLCVLEELVSGPSLLGYC <u>CSG</u>	14.5	0.6	1.5	0.4	2.3
H8	FHEKFYDWFDL.LSGSGSGSGSHLCVREEPFWGASLFGYC <u>CPG</u>	9.7	0.6	1.5	0.4	2.3

FIG. 44B-2

IGFR Binders with change in Cys

Clone	Sequence	E-Tag	IR	IGFR	IR/IGFR	IGFR/IR
F8 (X14)	HLCVLEELFWGASLFGYC _{SG}					
D8 (X6)	WLDQEWAVQCCEVYGRGCPS					
G1 (X4)	SHDNFYDWFVR.VSGSGSGSGSPLCVLGNCSG	11.3	2.8	10.6	0.3	3.8
E8 (X6)	FYDNFYHWFDR.VSGSGSGSGSHLCVLEERVCGASLFDYRSG	13.5	0.9	3.4	0.3	3.7
E3 (X10)	FHENFYDWFDRQVSGSGSGSGSHQCQVEERFWGASLCGYC _{SG}	15.9	1.9	6.7	0.3	3.5
F8 (X6)	FHGDFYDWFVR.VSGSGSGSGSHLCVLEELYC _{SG}	13.7	3.6	9.5	0.4	2.6
E1 (X4)	FQDNFYDWFVRQVSGSGSGSGSHRCVLEGC _{SG}	13.4	5.8	13.3	0.4	2.3
H12 (X4)	FHENFYDWFDRQVSGGSACLFYC _{SG}	9.8	3.9	8.5	0.5	2.2
H6 (X3)	VHESFYDWFVR.VAGSGSGSGSHLCDVD _{CSG}	11.5	4.8	9.6	0.5	2.0
E10 (X4)	FHENFYDWFDRQVSGSGGVLDERF.GACPSGYC _{SG}	10.6	5.1	8.9	0.6	1.8
F12 (X2)	FQENFYDWFVR.VSGDELSGGASQC _{SGSG}	10.6	7.0	9.6	0.7	1.4
F9 (X5)	SHESFYDWFVRQVSGSGSGSGSDL _{CVWEELCGGAPLVG.GSS}	16.0	9.9	13.3	0.7	1.3

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IGFR Binders with loss of F8

G7	FHANFYDWFVRQV.GSGSGSGSGSG	16.1	2.1	9.8	0.2	4.7
F2	YHENFYDWFVR.VSGSGSG	14.4	6.2	12.8	0.5	2.1
E7	FHENFYDWFVRQVSGSGSGSGG	15.4	7.8	14.0	0.6	1.8
E4	FPENFYDWFDRQVSGSGSGSGSGSG	16.4	13.4	15.8	0.8	1.2

IGFR Binders with loss of Cys in F8

F11	FHENFYDWFVRQVSGSGSGSGSHL.GSG	12.5	0.8	5.2	0.2	6.5
H7	.HENFYDWFVRQLSGSGSGSDGSHLFGYSGG	7.2	0.6	2.7	0.2	4.5
F7	FHENFYDWFDRQVSGSGSGSGGSPVTRGRTVLGGFSVRLLLW	15.0	2.7	10.9	0.2	4.1
G2	FPENFYDWFDRQVSGSGSGGASLFG.GSG	15.3	3.8	13.1	0.3	3.5
H4	FHDNFYDWFDRQVSGSGSGGSPFG.RSD	11.2	5.3	10.0	0.5	1.9

FIG. 44B-3

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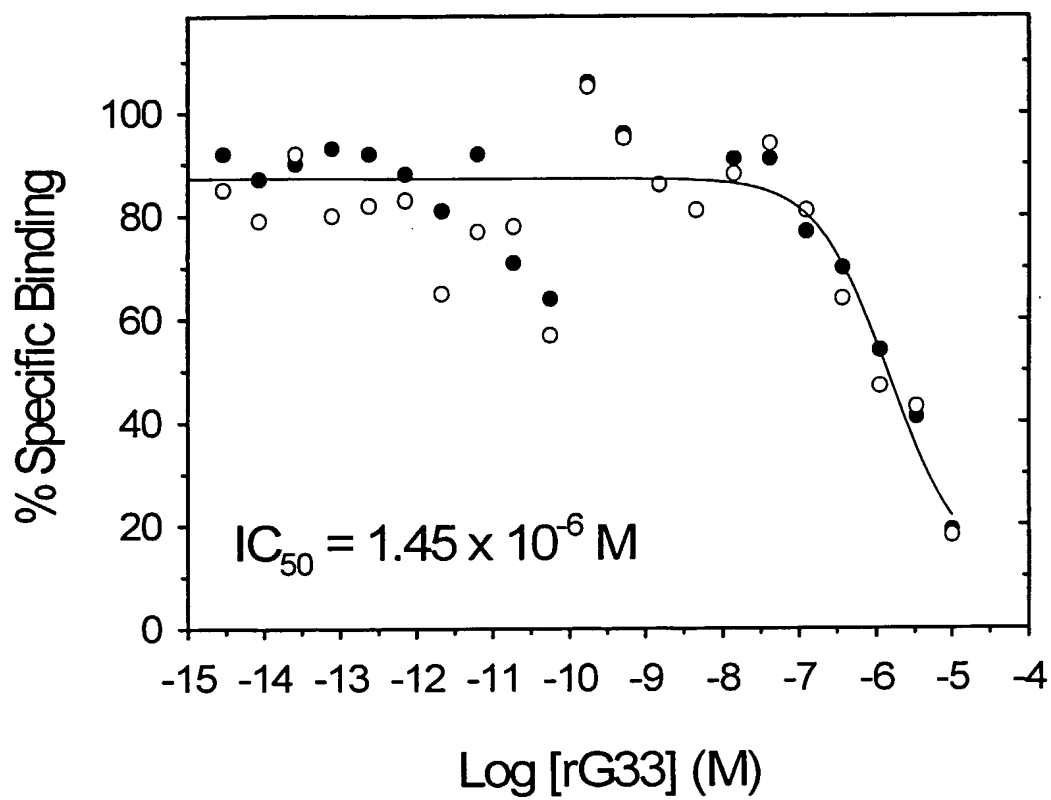


FIG. 45

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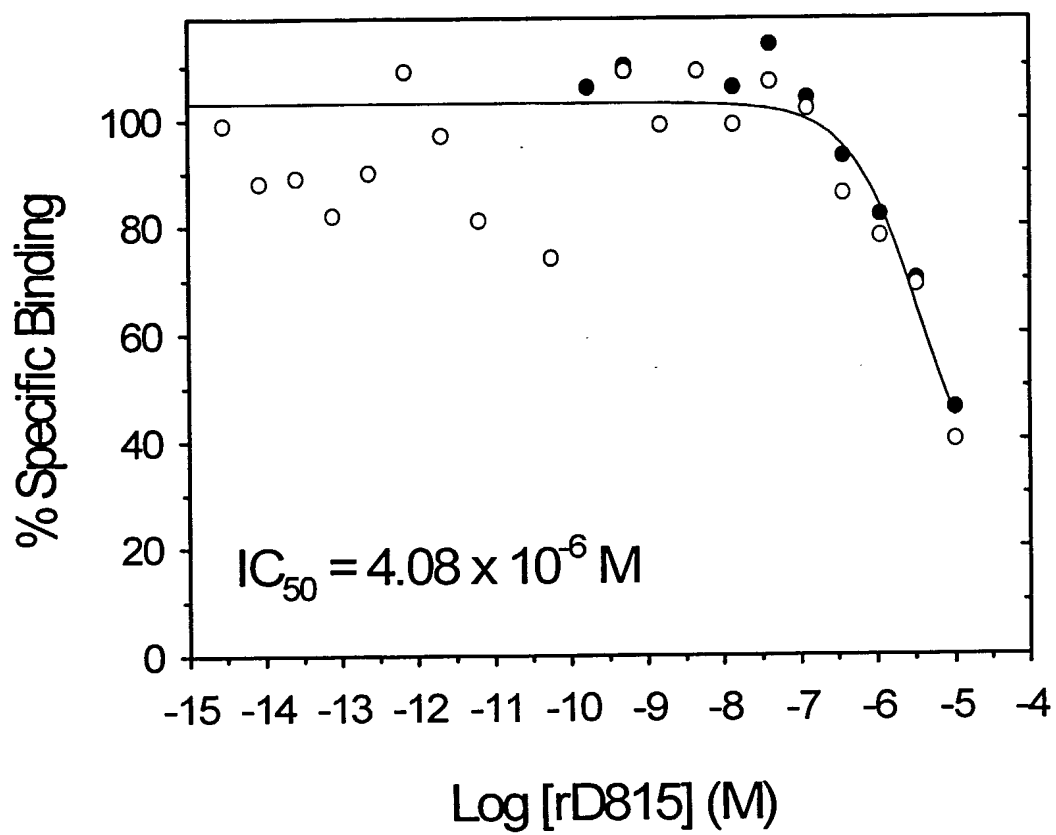


FIG. 46

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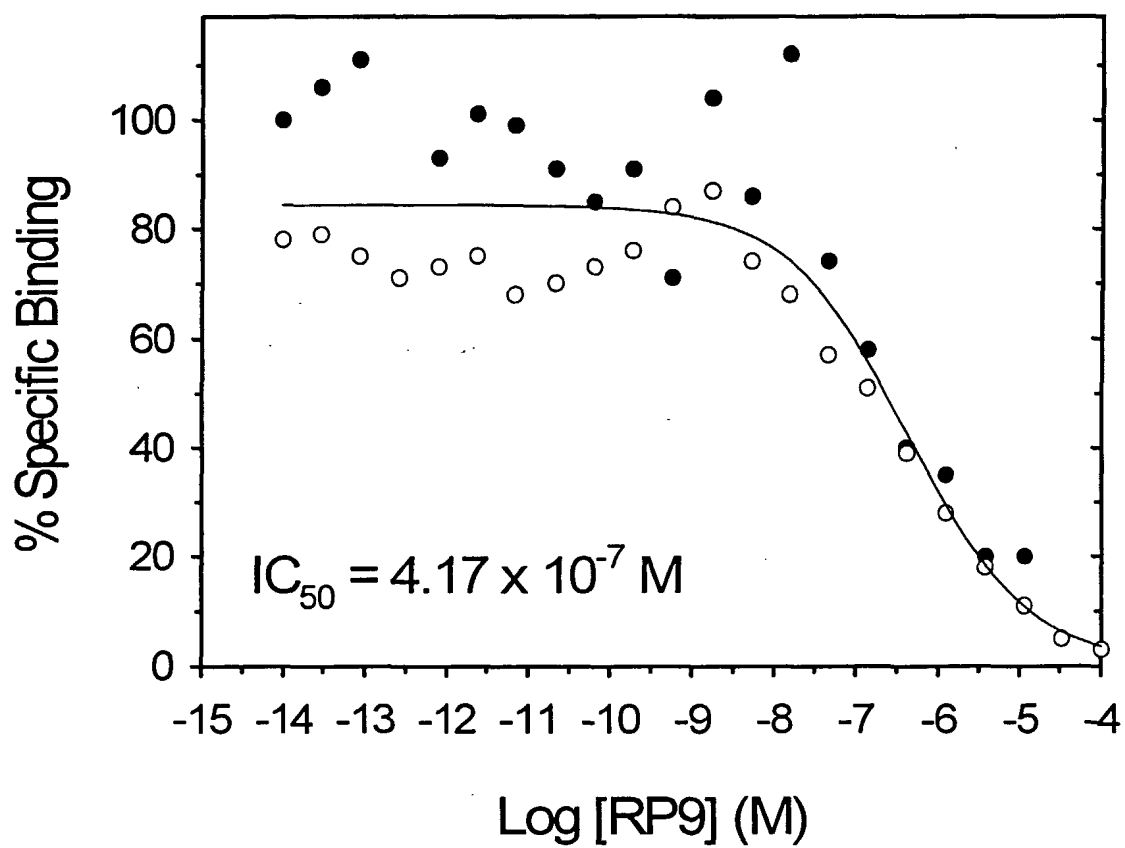


FIG. 47

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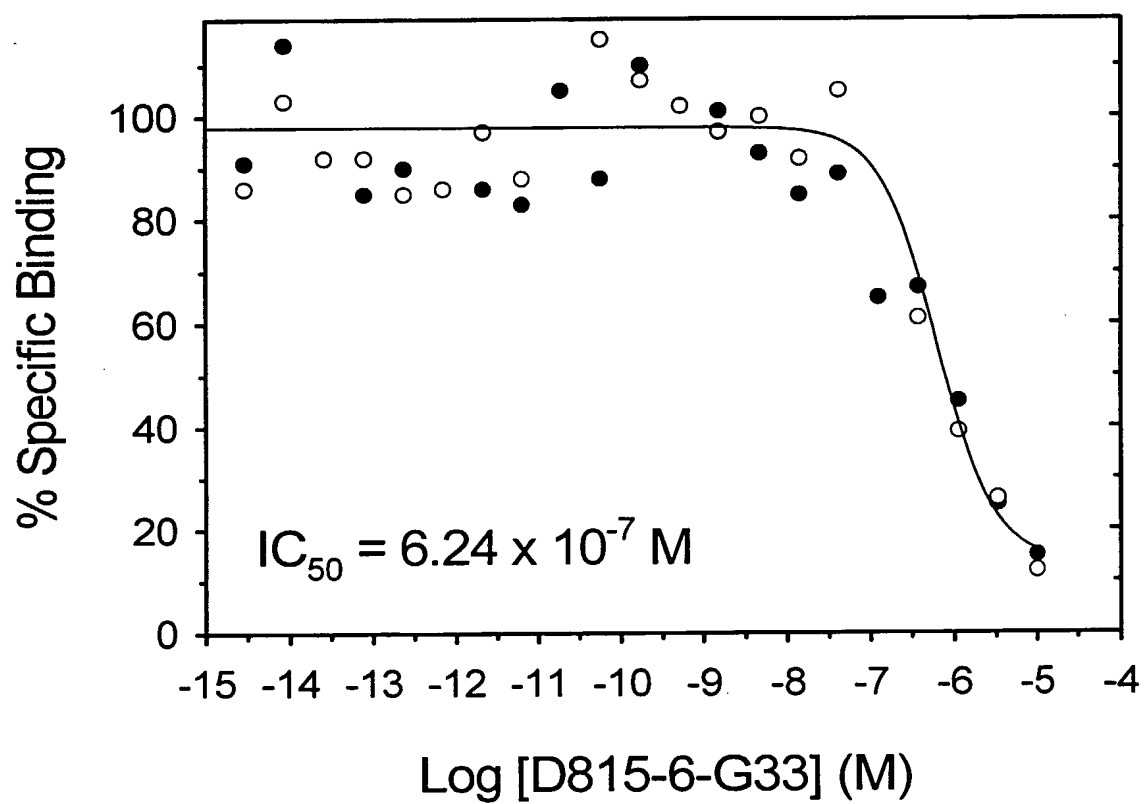


FIG. 48

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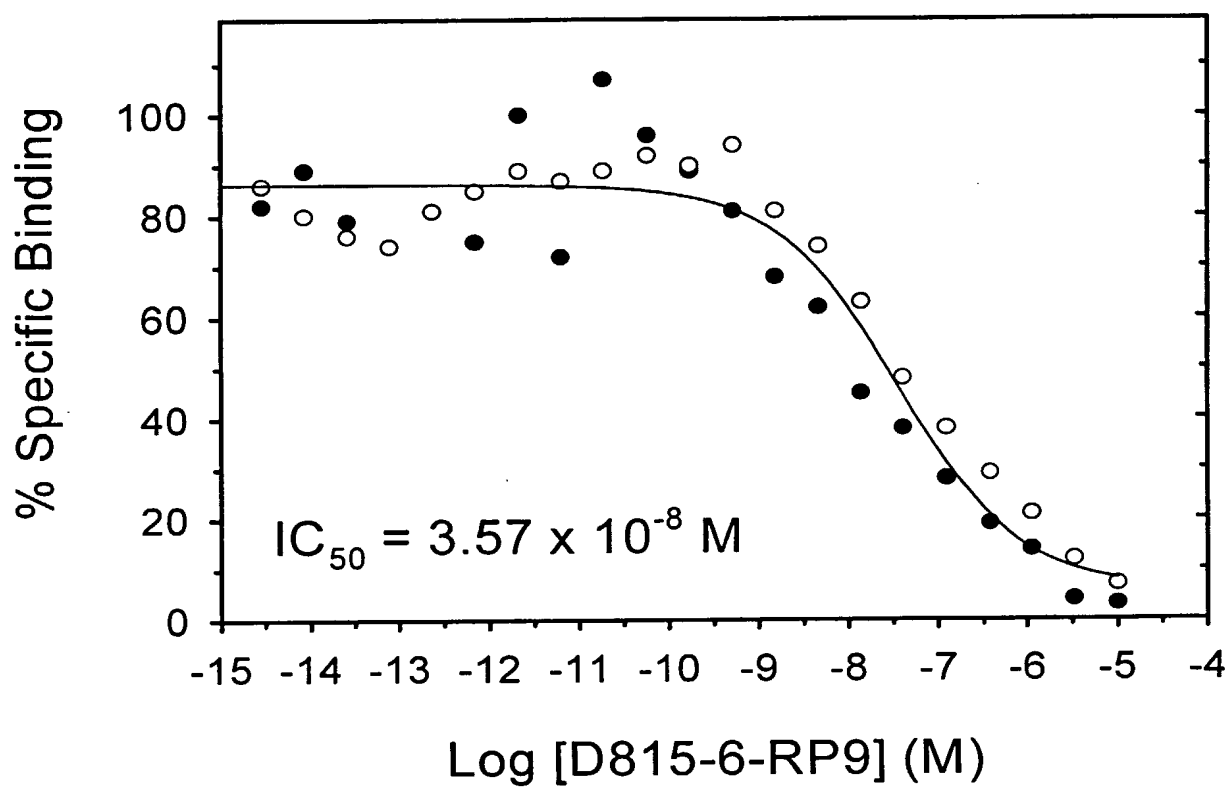


FIG. 49

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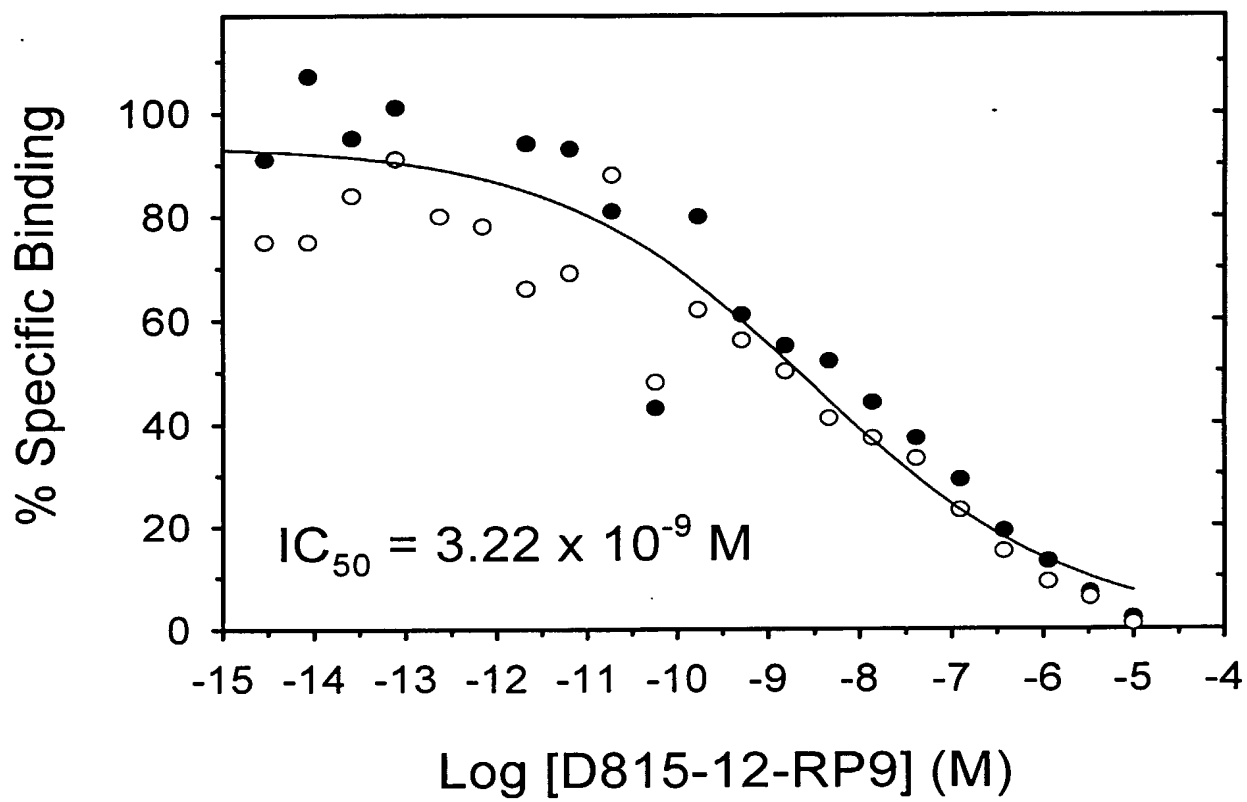


FIG. 50

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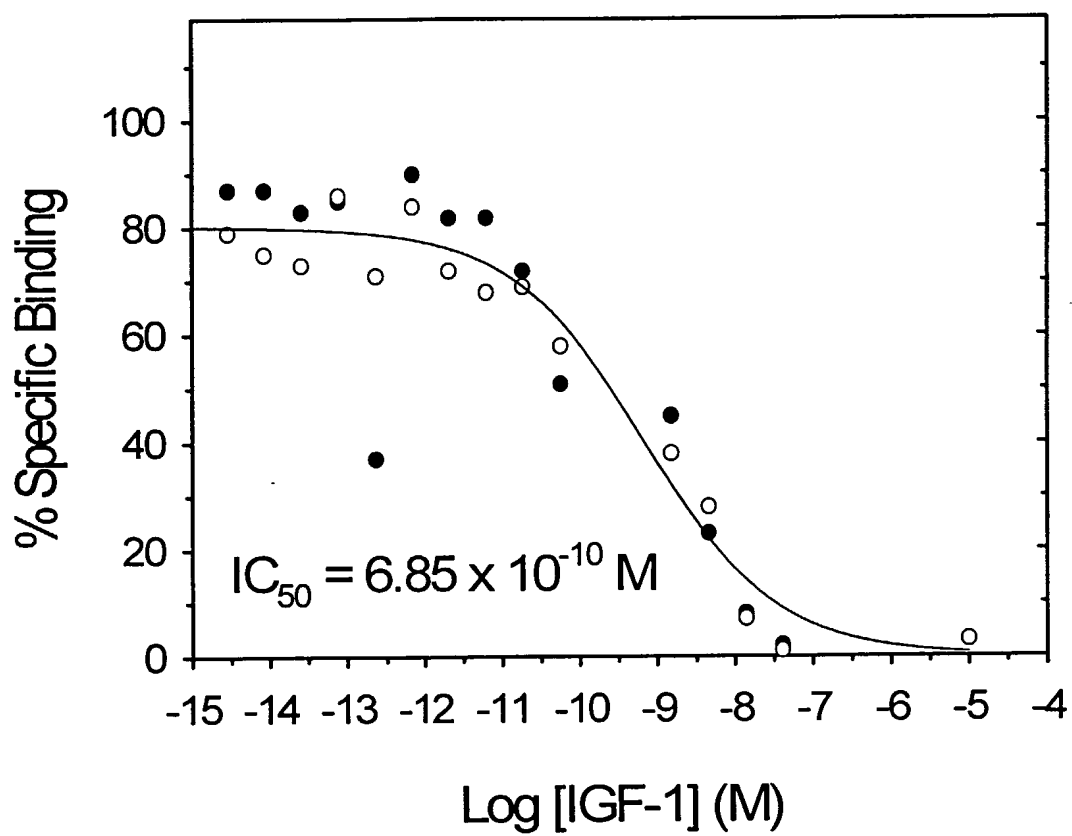


FIG. 51

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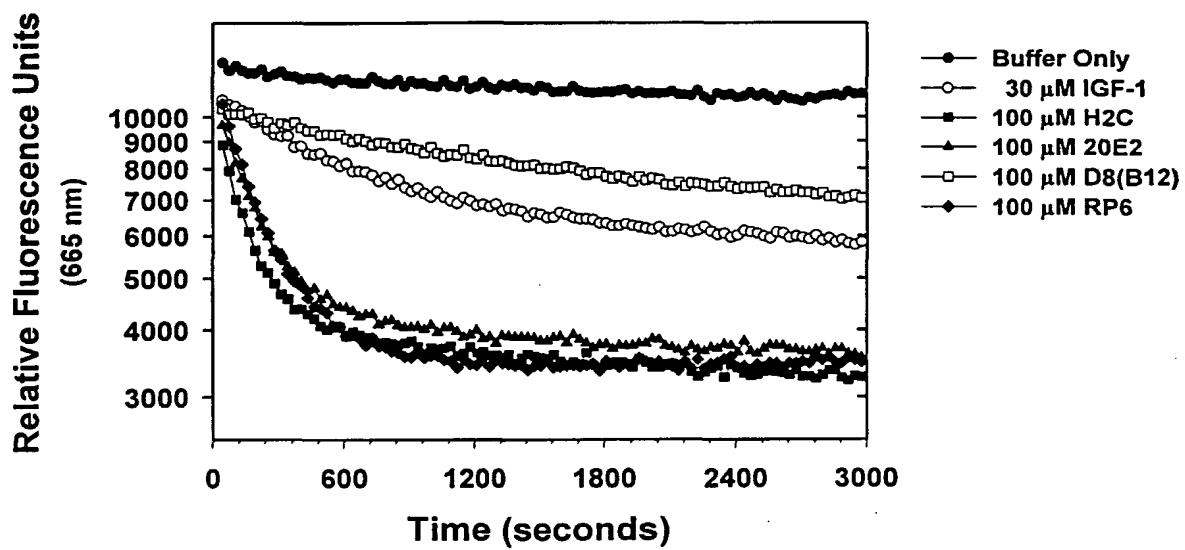


FIG. 52

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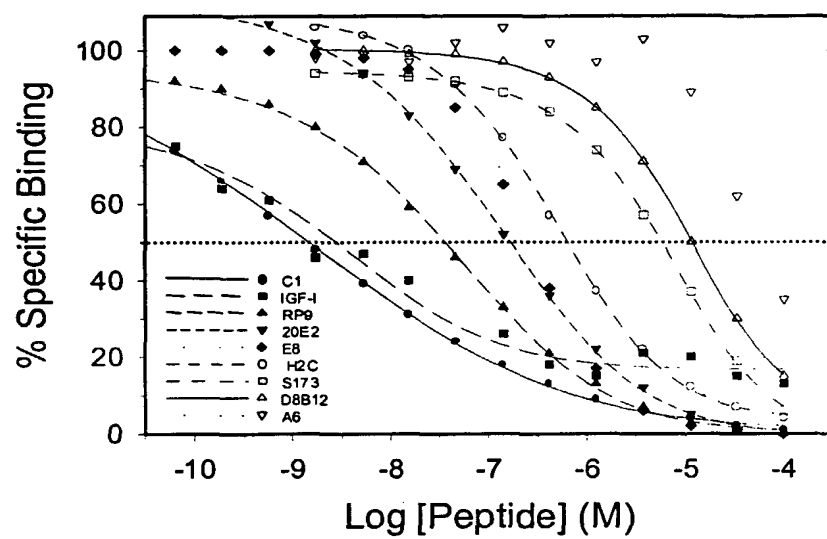


FIG. 53

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RP6 vs IGFR	Clone #	Sequence	Etag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
	Design	DYKDDDDKTFYSCLASLLTGTQPNRGPWRCR (FLAG)						
	RP6-IGFR-F9	TFYSCLAALLTDRPRPHGGPVRCR	13.4	9.8	1.1	9.1	0.1	
	RP6-IGFR-E9	SFYSCMASLLTGTQPCSGPRGWF	14.4	9.8	2.1	4.7	0.2	
	RP6-IGFR-H12	SFYSCLASLLTGTPEPCVRSQRL	7.8	9.8	3.7	2.7	0.4	
	RP6-IGFR-C11	TFYSCLSALVTGTRLPNRPWESCR	12.8	9.6	1.1	8.4	0.1	
	RP6-IGFR-G4	TFYSCLAACLVGTAEKCGCTWESR	12.7	9.1	3.1	2.9	0.3	
	RP6-IGFR-E7	AFYACMASLLSGSAMPGCCAGARS	14.1	8.7	2.1	4.2	0.2	
	RP6-IGFR-G8	TFYSCLASLVGTPLRNGVGDRCR	13.2	8.6	1.5	5.9	0.2	
	RP6-IGFR-B8	TFYSCLASLVACTPQPKGGACGGCH	11.5	8.3	1.8	4.6	0.2	
	RP6-IGFR-D11	AFYSCLATLASGTRLCNRGLWDSGR	15.7	7.8	2.7	2.9	0.3	
	RP6-IGFR-D8	TFYSCLDLVQ . PAASDCAVGTR	12.1	7.5	1.7	4.6	0.2	
	RP6-IGFR-F8 ₄	IFYLCASLLNGTQPNCGP . ELSR	11.7	7.5	1.9	4.0	0.3	
	RP6-IGFR-B6	RFYACLAALLNGTPRLSGGPGILCR	13.4	6.9	1.0	6.6	0.2	
	RP6-IGFR-E4	NFYGCLAALLSGTPHSQRGAWEGR	9.4	6.8	1.0	7.0	0.1	
	RP6-IGFR-C6	YFYSCLASLVTDSELSKRGPCGPR	13.4	6.0	1.2	5.0	0.2	
	RP6-IGFR-B9	TFYSCMAAL . NGTREPDRCSRGGSR	10.6	5.9	2.2	2.7	0.4	
	RP6-IGFR-H6	NFYSCLA . LVTGPAQPNSTQWPVCR	9.4	5.7	1.3	4.4	0.2	
	RP6-IGFR-F11 ₆	TFYNCIASLLNGTPESNGVQ . DFCR	13.3	4.1	3.4	1.2	0.8	
	RP6-IGFR-A7	TFYSCLASLVSGPQPNRGRWEHCR	7.2	4.0	1.0	4.0	0.3	
	RP6-IGFR-H5	TFYSCIGSLLTGTPEGGPWESCR	6.9	2.9	0.9	3.4	0.3	
	RP6-IGFR-A12	IFYSCLASLLTGSAPNRCVGERGR	3.1	2.7	1.0	2.6	0.4	
	RP6-IGFR-G6	TFYSCMASLLASTPHTPRGW . CGG	14.2	15.3	1.6	9.7	0.1	
	RP6-IGFR-G3	HFYSCLAQLLAGTPKSTRGPGERCR	13.8	15.0	1.0	15.0	0.1	
	RP6-IGFR-B12	RFYSCIASLMRGTPLASRQQRVICR	12.7	14.9	1.0	14.6	0.1	
	RP6-IGFR-C5	TFYSCIAADLLASSERYRGRDRCR	14.2	14.9	1.1	13.2	0.1	
	RP6-IGFR-F4	TFYSCIASLLTGTQNRNGSGDRCR	11.8	14.6	2.8	5.2	0.2	
	RP6-IGFR-G7	TFYSCIAFLVGTAEQKRGSWERCR	14.3	14.5	1.3	10.9	0.1	
	RP6-IGFR-C10	AFYSCIASLLSGAAQCNRE . ERSR	16.5	14.0	6.6	2.1	0.5	
	RP6-IGFR-E5	TFYSCIASLLSGPPQGRGLRKRC	14.5	13.8	1.8	7.8	0.1	
	RP6-IGFR-D5	NFYTCISALLAGPPLPNRCTGERGR	14.5	13.8	3.9	3.5	0.3	
	RP6-IGFR-E12	PFYSCIAALATGAPESNRGGWRCR	12.9	13.6	3.4	4.0	0.3	
	RP6-IGFR-B11	NFYSCISALLSGAPQPHGSSWERC	14.7	13.4	1.1	12.4	0.1	
	RP6-IGFR-G10	TFYSCIAALVTGTPHCGTGERNP	11.9	13.4	4.0	3.4	0.3	
	RP6-IGFR-E10	TFYSCISALLIGTPQPNRPGDGR	14.1	12.8	1.0	13.4	0.1	
	RP6-IGFR-D6	TFYSCIASLLTGTGPHKGWRCR	12.3	12.7	0.9	13.5	0.1	
	RP6-IGFR-F6	TFYSCMASLMTGPPPNRKGWCWR	13.1	12.5	2.0	6.3	0.2	
	RP6-IGFR-F5	TFYSCIASLVMTGPTLTNRQWGERGR	12.5	12.3	5.7	2.2	0.5	
	RP6-IGFR-C9	SFYSCLA . LVTGSRGTGRGPSECH	11.2	12.0	1.1	10.8	0.1	
	RP6-IGFR-B3	TFYSCIGSLLAG . SPGRGLWEGR	11.7	10.8	6.5	1.7	0.6	

FIG. 54A

Clone #	Sequence	Etag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
RP6-IGFR-D7	EFYSCMAALLRVSLKQSSASWGRCR	14.6	10.3	1.8	5.6	0.2	
RP6-IGFR-H7	TFYSCLA.LVTGSPQPNRCPWGRGR	9.8	10.1	1.4	7.1	0.1	
RP6-IGFR-F10	VFYSCMASLLAGVPLTNCGPGR.R	10.9	10.1	4.6	2.2	0.5	
RP6-IGFR-C3	TFYECLASLLADTPQPNPGPWGRCR	10.8	1.3	0.9	1.4	0.7	
RP6-IGFR-B10	IFEYCLAPRLTGSPQPYRYPWGRGR	10.6	1.3	1.3	1.0	1.0	
RP6-IGFR-C12	TFYSCLD.LLTGGPQPNRGADHCR	1.1	1.2	0.2	6.2	0.2	
RP6-IGFR-A5	TFSSCIPPLMSDTSHPSRGQCVRCR	2.3	1.1	1.0	1.0	1.0	

FIG. 54B

RP9NPB25 VS IGFR

Clone #	Design	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
RP9NPB25 - IGFR - F11		GSLDESFYDWFERQLGXXXXXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--	
RP9NPB25 - IGFR - E7		GSLDESFYDWFERQLGAVVGRQDGGPITRDVGGDGRGVNV	10.9	15.0	11.6	1.3	0.8	
RP9NPB25 - IGFR - E6		GSLDESFYDWFERQLGADTKSPRWGSGTREHQARGGAPGR	12.6	14.9	13.7	1.1	0.9	
RP9NPB25 - IGFR - A8		GSLDESFYDWFERQLGSDRNPGLRSALDRAGDSPILIS	13.0	14.5	13.2	1.1	0.9	
RP9NPB25 - IGFR - G12		GSLDESFYDWFERQLGSDRNPGLRSGRRNLEGIR	11.8	14.2	10.9	1.3	0.8	
RP9NPB25 - IGFR - C11		GSLDESFYDWFERQLGSHVDETRAEALPQISQQQANGVQ	10.8	14.2	13.0	1.1	0.9	
RP9NPB25 - IGFR - C10		GSLDESFYDWFERQLGRGAVDEGRSSAVGAVRRDDQR	12.4	14.2	12.5	1.1	0.9	
RP9NPB25 - IGFR - F8		GSLDESFYDWFERQLGLVMGEGRGAEIRDRHCLSVGSACLE	12.8	14.1	10.3	1.4	0.7	
RP9NPB25 - IGFR - B9		GSLDESFYDWFERQLGVAPGRVAVVGRGHGDASGGRRRTG	12.6	14.0	9.6	1.5	0.7	
RP9NPB25 - IGFR - E4		GSLDESFYDWFERQLGLSADQSQTPAVRGAPRRGL	11.5	13.8	8.8	1.6	0.6	
RP9NPB25 - IGFR - C6		GSLDESFYDWFERQLGNGGNDGELRESRASRGSIHKNKA	11.4	13.5	12.5	1.1	0.9	
RP9NPB25 - IGFR - E11		GSLDESFYDWFERQLGKARVVLGQEVCELESSEGSCI	15.0	13.4	7.8	1.7	0.6	
RP9NPB25 - IGFR - G11		GSLDESFYDWFERQLGTLAQVPSAPTRAAVGGSHGEQKVG	11.5	13.4	11.7	1.1	0.9	
RP9NPB25 - IGFR - B12		GSLDESFYDWFERQLGVKGQGGVSVGVGPCTAIRRNLCG	9.8	13.3	3.6	3.7	0.3	
RP9NPB25 - IGFR - B12		GSLDESFYDWFERQLGQHQSGLGRGGRGASFSSDGLNKG	10.6	13.3	10.1	1.3	0.8	
RP9NPB25 - IGFR - B8		GSLDESFYDWFERQLGRGRSGTGGPVLSDHQMVAGSRVATQ	10.8	13.2	12.0	1.1	0.9	
RP9NPB25 - IGFR - D10		GSLDESFYDWFERQLGVKGKGGHVNRTRKPLGSRGDGVAGY	11.1	13.1	8.5	1.5	0.6	
RP9NPB25 - IGFR - F9		GSLDESFYDWFERQLGDPWSEVRVGRKEGGRVGFVGHVGV	11.4	13.0	11.9	1.1	0.9	
RP9NPB25 - IGFR - G5		GSLDESFYDWFERQLGSGHIGRQDCLAAAGDPRRTTC	11.5	12.8	12.1	1.1	0.9	
RP9NPB25 - IGFR - G4		GSLDESFYDWFERQLGSAVSSVPAGHKDISRQDVGEKSRAG	11.1	12.7	11.0	1.2	0.9	
RP9NPB25 - IGFR - D4		GSLDESFYDWFERQLGSHKRCSSVRTGLVADCADGGLGY	11.0	12.6	11.3	1.1	0.9	
RP9NPB25 - IGFR - D12		GSLDESFYDWFERQLGSWDARACQSGDQGVYSETDLGR	10.0	12.4	8.7	1.4	0.7	
RP9NPB25 - IGFR - B3		GSLDESFYDWFERQLGGLAHEGRNLGSGSPSDAGGKHDEVA	10.0	12.3	9.4	1.3	0.8	
RP9NPB25 - IGFR - E8		GSLDESFYDWFERQLGTAEAGVGHNLGRGQ	10.8	12.2	6.7	1.8	0.5	
RP9NPB25 - IGFR - E3		GSLDESFYDWFERQLGVSAKPSGRVKKDHPDTGTNGRLVRD	10.5	12.2	12.6	1.0	1.0	
RP9NPB25 - IGFR - B11		GSLDESFYDWFERQLGMQGTAINRTYGPPEEAVRSGGYPL	9.8	12.1	8.6	1.4	0.7	
RP9NPB25 - IGFR - E12		GSLDESFYDWFERQLGAAGSEADGGSTTVRPSMLPRTQS	9.7	12.0	10.0	1.2	0.8	
RP9NPB25 - IGFR - B2		GSLDESFYDWFERQLGSESTRRGGWHPVRNSALSERG	10.0	12.0	10.9	1.1	0.9	
RP9NPB25 - IGFR - F5		GSLDESFYDWFERQLGRHDEIHEAGRQGAULTDSGVRPHV	10.3	11.9	9.7	1.2	0.8	
RP9NPB25 - IGFR - B5		GSLDESFYDWFERQLGESRYERRPVRGRSGMGLVDSGLSD	10.2	11.9	10.7	1.1	0.9	
RP9NPB25 - IGFR - F4		GSLDESFYDWFERQLGLAVRSDRAGRNTPRVYGTSTRSAVAT	10.5	11.8	11.1	1.1	0.9	
RP9NPB25 - IGFR - D7		GSLDESFYDWFERQLGAPERVTGSRGLNRDLQGRDAYWR	9.9	11.7	9.9	1.2	0.8	
RP9NPB25 - IGFR - F1		GSLDESFYDWFERQLGHVGLPLSRGACTRVGTSQIGVAGP	10.1	11.6	9.7	1.2	0.8	
RP9NPB25 - IGFR - D3		GSLDESFYDWFERQLGGVVEDGGKGRSRSRKPGKLPKSG	9.9	11.6	11.4	1.0	1.0	
RP9NPB25 - IGFR - G3		GSLDESFYDWFERQLGSRADAPMEHPARWEDGRVGPGLQD	11.1	11.5	7.8	1.5	0.7	
RP9NPB25 - IGFR - A6		GSLDESFYDWFERQLGRAGPENGVSQPGAAVGMGGSIG	9.6	11.5	11.2	1.0	1.0	
RP9NPB25 - IGFR - G9		GSLDESFYDWFERQLGNGEGVGHPPNMQVIGSAQRAKDS	10.2	11.4	8.0	1.4	0.7	

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FIG. 55A

Clone #	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
RP9NPB25-IGFR-F3	GSLDESFYDWFERQLGA.RTSPPISSCDRDRSGNMRRDC	9.8	11.4	10.5	1.1	0.9	122/163
RP9NPB25-IGFR-F6	GSLDESFYDWFERQLGAGRERGSLDRERQSGIGGQKGM	9.8	11.3	10.1	1.1	0.9	
RP9NPB25-IGFR-G7	GSLDESFYDWFERQLGRRQPDSESLDQAGWVSRIHKGGPK	10.2	11.3	10.2	1.1	0.9	
RP9NPB25-IGFR-A5	GSLDESFYDWFERQLGKPLTSAGRNHNAGCETLGSCLMDRS	8.9	11.1	4.6	2.4	0.4	
RP9NPB25-IGFR-F2	GSLDESFYDWFERQLGQSVDSMPVDHPQSMQRGRVFGG	10.1	10.7	5.3	2.0	0.5	
RP9NPB25-IGFR-D1	GSLDESFYDWFERQLGGDSAGTARGSGRGLSAGADGAIVG	8.6	10.7	6.7	1.6	0.6	
RP9NPB25-IGFR-C5	GSLDESFYDWFERQLGGVGIGPVPDRDQGFDSGGGAESSG	10.4	10.7	9.0	1.2	0.8	
RP9NPB25-IGFR-C3	GSLDESFYDWFERQLGTSLAFLRGGDEGWRGGQAPSAANR	11.5	10.6	7.4	1.4	0.7	
RP9NPB25-IGFR-F10	GSLDESFYDWFERQLGARVDGLSARGATCPTSGCGVLQPP	8.9	10.4	7.2	1.5	0.7	
RP9NPB25-IGFR-B4	GSLDESFYDWFERQLGPEDRPVGGAIVGQGFNGVVTIDSA	9.6	10.3	7.2	1.4	0.7	
RP9NPB25-IGFR-E9	GSLDESFYDWFERQLGTYSQGVDRDTSRQ	11.3	10.3	7.1	1.4	0.7	
RP9NPB25-IGFR-E5	GSLDESFYDWFERQLGNSVTDRAHATGLAGKQDSVDIEVT	11.3	10.1	6.5	1.6	0.6	
RP9NPB25-IGFR-B7	GSLDESFYDWFERQLGLRGQDAVGRDQTGFRDQGRGSHGR	8.6	10.1	9.5	1.1	0.9	
RP9NPB25-IGFR-G10	GSLDESFYDWFERQLGLPQSGVPGSSGGRGRTSSSMRHGVS	8.8	9.9	8.8	1.1	0.9	
RP9NPB25-IGFR-C4	GSLDESFYDWFERQLGDGRLGCQGYRAEVDMEGQPRGGCG	9.8	9.8	8.6	1.1	0.9	
RP9NPB25-IGFR-D8	GSLDESFYDWFERQLGTVRGESVVRDAQSPVDSPPGGVARS	8.9	9.6	6.5	1.5	0.7	
RP9NPB25-IGFR-D11	GSLDESFYDWFERQLGAVGVDVGGYREAPRGWGARWLQAD	8.0	9.4	8.2	1.2	0.9	
RP9NPB25-IGFR-A4	GSLDESFYDWFERQLGVVVRREGNEV	7.7	9.3	7.7	1.2	0.8	
RP9NPB25-IGFR-A10	GSLDESFYDWFERQLGVDVGGRRRGGG	7.5	9.2	9.0	1.0	1.0	
RP9NPB25-IGFR-A11	GSLDESFYDWFERQLGSKPVLGRGQERRDKPGVEEGGAA	7.3	9.0	7.4	1.2	0.8	
RP9NPB25-IGFR-B1	GSLDESFYDWFERQLGARVGMGRGPMLDPRMGPGETQVPS	7.3	8.0	5.9	1.4	0.7	
RP9NPB25-IGFR-D6	GSLDESFYDWFERQLGQTAPVGDA [~] SRTKGALQ [~] PVGGWYVRG	9.0	7.7	4.7	1.6	0.6	
RP9NPB25-IGFR-G8	GSLDESFYDWFERQLGHVPRAAHVGAQLGSRGPGSLDSLGV	6.0	6.8	4.0	1.7	0.6	
RP9NPB25-IGFR-A9	VEWQLVTVAAAGEGPNLLGVSYFSCLESLVNGGAERSDQWEGCR	9.4	5.3	1.5	3.5	0.3	
RP9NPB25-IGFR-C12	GSLDESFYDWFERQLGAGDRGQSR [~] TTCTMQRNREGAAESC [~] TV	0.9	1.0	0.9	1.1	0.9	

FIG. 55B

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RP30-NPB20-vs-IGFR

Clone #	Design	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
RP30NPB20-IGFR-E9		SFYSCLESVNGGAERSDQWEGCRXXXXXXXXXXXXXXXXXXXX	--	--	--	--	--	--
RP30NPB20-IGFR-E11		SFYSCLESVNGGAKRSDQWEGCRSSGWLGSFSGPRQANSQSG	17.7	19.2	1.1	17.3	0.1	1.1
RP30NPB20-IGFR-G12		SFYSCLESVNGGAERSDQWEGCRGQGGSLTMGFPGLQESGV	17.1	18.5	1.3	14.4	0.1	1.1
RP30NPB20-IGFR-E8		SFYSCLESVNGGAERSDQWEGCRKRHSASSRGEDRLVGVGSA	15.7	17.5	1.8	9.8	0.1	1.1
RP30NPB20-IGFR-E5		SFYSCLESVNGGAERSDQWEGCRAGGKRMGIDAEAGFWGVP	17.4	17.1	1.2	14.5	0.1	1.1
RP30NPB20-IGFR-F11		SFYSCLESVNGGAERSDQWEGCRSGQSVRLNNGGIGRFKVP	15.9	17.1	1.8	9.7	0.1	1.1
RP30NPB20-IGFR-F7		SFYSCLESVNGGAERSDQWEGCRFSTSRADSAGPGVPWGS	16.7	14.9	1.6	9.5	0.1	1.1
RP30NPB20-IGFR-F6		SFYSCLESVNGGAERSDQWEGCRAPGHAWSVGHVDRDSTQ	14.9	14.9	2.0	7.5	0.1	1.1
RP30NPB20-IGFR-F12		SFYSCLESVNGGAERSDQWEGCRAGGVQRMAGFSQSNRMH	14.2	14.8	3.3	4.5	0.2	1.1
RP30NPB20-IGFR-D2		SFYSCLESVNGGAERSDQWEGCRARNRDAALDGAADLIQGMQ	12.7	14.7	1.0	14.3	0.1	1.1
RP30NPB20-IGFR-D3		SFYSCLESVNGGAERSDQWEGCRRGWVEDHEDGGMLGSLAQ	13.1	14.7	2.1	7.0	0.1	1.1
RP30NPB20-IGFR-D5		SFYSCLESVNGGAERSDQWEGCRGTGGAAPRGATWIGTPLQ	12.8	14.6	6.5	2.3	0.4	1.1
RP30NPB20-IGFR-H2		SFYSCLESVNGGAERSDQWEGCRVEGVHGLEPGGSLHGGL	14.8	14.4	2.9	4.9	0.2	1.1
RP30NPB20-IGFR-E6		SFYSCLESVNGGAERSDQWEGCRGVPGVVQASQWAEQVE	13.6	14.1	1.4	10.3	0.1	1.1
RP30NPB20-IGFR-B3		SFYSCLESVNGGAERSDQWEGCRGSGITGDLRTVSGRDAAI	15.3	13.7	1.5	9.2	0.1	1.1
RP30NPB20-IGFR-F1		SFYSCLESVNGGAERSDQWEGCRGRIAP	12.5	13.4	1.5	8.8	0.1	1.1
RP30NPB20-IGFR-E3		SFYSCLESVNGGAEDQWEGCRGHTSFYRHLGRTGGSV	12.7	13.2	1.1	12.4	0.1	1.1
RP30NPB20-IGFR-E12		SFYSCLESVNGGAERSDQWEGCRTRAGQVRYAASGDPLRAA	13.6	13.0	1.2	11.0	0.1	1.1
RP30NPB20-IGFR-C10		SFYSCLESVNGGAERSDQWEGCRAPGDFVFGGRVAQQGI	10.4	12.6	0.8	16.3	0.1	1.1
RP30NPB20-IGFR-E4		SFYSCLESVNGGAERSDQWEGCRVGRLOGGPALSM	13.4	12.6	1.9	6.6	0.2	1.1
RP30NPB20-IGFR-F2		SFYSCLESVNGDAERSDQWEGCHGASSRGVRQTTGGRSWY	14.2	12.1	1.0	12.9	0.1	1.1
RP30NPB20-IGFR-D8		SFYSCLESVNGGAERSDQWEGCRGGLRSGWDMRETAGPLGA	15.7	12.0	1.1	10.5	0.1	1.1
RP30NPB20-IGFR-G3		SFYSCLESVNGGAERSDQWEGCRPAPRAGGRGIIQSGGARV	11.2	11.8	2.5	4.7	0.2	1.1
RP30NPB20-IGFR-F10		SFYSCLESVNGGAERSDQWEGCRRGFDAGMGWSTAGSRGVS	10.0	11.8	3.3	3.6	0.3	1.1
RP30NPB20-IGFR-F9		SFYSCLESVNGGAERSDQWEGCRSGALSGLFNGLPVLADHG	13.5	11.6	0.8	13.8	0.1	1.1
RP30NPB20-IGFR-G11		SFYSCLESVNGGAERSDQWEGCRSGQVVSAGGPGASSAAGG	13.1	11.6	1.0	11.2	0.1	1.1
RP30NPB20-IGFR-B12		SFYSCLESVNGGAERSDQWEGCRGWDIVLDGRVQNGSAGHI	13.2	11.3	1.0	10.8	0.1	1.1
RP30NPB20-IGFR-G7		SFYSCLESVNGGAERSDQWEGCRGSDVGLVPGPGGSPVLS	10.6	11.0	2.2	5.0	0.2	1.1
RP30NPB20-IGFR-A7		SFYSCLESVNGGAERSDQWEGCRSYHGHREWPLQDGGDDSVI	10.4	11.0	2.5	4.4	0.2	1.1
RP30NPB20-IGFR-G6		SFYSCLESVNGGAERSDQWEGCRGGIGGAVGTGRTQVQIR	11.1	10.8	1.8	6.0	0.2	1.1
RP30NPB20-IGFR-C4		SFYSCLESVNGGAERSDQWEGCRFRPTDSGEKGTAPAGGI	9.9	10.8	2.0	5.4	0.2	1.1
RP30NPB20-IGFR-G8		SFYSCLESVNGGAERSDQWEGCRPWAPSADVEIFGCGNVC	10.9	10.8	2.5	4.4	0.2	1.1
RP30NPB20-IGFR-G4		SFYSCLESVNGGAERSDQWEGCRDHFEGGGLAQDIGSRPGG	12.4	10.5	1.4	7.4	0.1	1.1
RP30NPB20-IGFR-H12		SFYSCLESVNGGAERSDQWEGCRGGLSAGPYGGGSAVALEGGN	11.4	10.4	1.9	5.5	0.2	1.1
		SFYSCLESVNGGAERSDQWEGCRAGGVVVRNVRPGRIRPRD	9.5	10.0	1.4	7.1	0.1	1.1
			12.0	10.0	2.2	4.6	0.2	1.1

FIG. 56A

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Clone #	Sequence	E-Tag	IGFR	IR	IGFR/IR	IR/IGFR	SEQ ID NO:
RP30NPB20-IGFR-D10	SFYSCLESLVNGGAERSDQWEGCRRRQAPGGVFRGGGRASAL	11.3	9.8	3.0	3.3	0.3	
RP30NPB20-IGFR-A5	SFYSCLESLVNGGAERSDQWEGCRTSGQQLGSRKTTSEGAVHA	14.2	9.5	1.5	6.5	0.2	
RP30NPB20-IGFR-A11	SFYSCLESLVNGGAERSDQWEGCRGFGVGVAVSGPGTTPAGSV	8.8	9.5	1.6	5.7	0.2	
RP30NPB20-IGFR-D7	SFYSCLESLVNGGAERSDQWEGCRRASVVRGHDQATFGYGVGGT	12.6	9.5	2.4	4.0	0.3	
RP30NPB20-IGFR-C3	SFYSCLESLVNGGAERSDQWEGCRIGRRAPGHQVQAQTHLRGV	12.9	9.4	1.9	5.0	0.2	
RP30NPB20-IGFR-C11	SFYSCLESLVNGGAERSDQWEGCRRFTRDGLVGLQGSSEGLV	9.9	9.2	1.2	7.6	0.1	
RP30NPB20-IGFR-G9	SFYSCLESLVNGGAERSDQWEGCRSSSSGAPWNGWAGFLHGN	10.0	9.2	3.3	2.8	0.4	
RP30NPB20-IGFR-D1	SFYSCLESLVNGGAERSGRWEGCRDVGKTPDSHEVARSELVV	7.6	9.2	3.3	2.7	0.4	
RP30NPB20-IGFR-C5	SFYSCLESLVNGGAERSDQWEGCRGNAGQPGRGAGHRRGA	9.4	9.2	3.8	2.4	0.4	
RP30NPB20-IGFR-H6	SFYSCLESLVNGGAERSDQWEGCRWLGRRSSGMSVGTGARLSS	10.6	8.6	1.1	7.9	0.1	
RP30NPB20-IGFR-D9	SFYSCLESLVNGGAERSDQWEGCRGVGPPFTNAPEVIRQGGGIS	7.7	8.6	1.1	7.5	0.1	
RP30NPB20-IGFR-D12	SFYSCLESLVNGGAERSDQWEGCRRRGKELSGAYQWKGAPLSWL	8.2	8.6	1.2	7.3	0.1	
RP30NPB20-IGFR-C7	SFYSCLESLVNGGTERSDDQWEGCRPVFGSAGHQDSTLAGVE	8.2	8.6	3.0	2.9	0.3	
RP30NPB20-IGFR-F6 ₂	SFYSCLESLVNGGAERSDQWEGCRTSIPGGGLKPRGSPGGALPI	17.9	8.5	1.4	6.1	0.2	
RP30NPB20-IGFR-C6	SFYSCLESLVNGGAERSDQWEGCRGSLWQVGRVLMGVSS	8.4	8.4	3.1	2.7	0.4	
RP30NPB20-IGFR-D4	SFYSCLESLVNGGAERSDQWEGCRSRQGLPGMEEVSGPTSGNH	15.1	8.3	2.0	4.2	0.2	
RP30NPB20-IGFR-H3	SFYSCLESLVNGGAERSDQWEGCRGARRQWPAGKDPSSRGDVP	9.1	8.2	1.5	5.5	0.2	
RP30NPB20-IGFR-B8	SFYSCLESLVNGGAERSDQWEGCRKDKGVWASSVPPRTQRRGH	6.8	7.9	2.4	3.3	0.3	
RP30NPB20-IGFR-G5	SFYSCLESLVNGGTERSDDQWEGCRAAQAFLGESGMNGVSWYGEQ	14.7	7.5	1.4	5.3	0.2	
RP30NPB20-IGFR-B7	SFYSCLESLVNGGAERSDQWEGCRQKVLRSRPFVLEGADTGT	10.3	7.4	2.0	3.6	0.3	
RP30NPB20-IGFR-B10	SFYSCLESLVNGGAERSDQWEGCRTTGLWGGDPPRRSAGIAF	9.0	7.4	3.1	2.4	0.4	
RP30NPB20-IGFR-F3	SFYSCLESLVNGGAERSDQWEGCRGSLWLRADWIGRGGRTQPDG	12.0	6.8	2.4	2.8	0.4	
RP30NPB20-IGFR-B6	SFYSCLESLVNGGAERSDQWEGCRQGRFFQFSRGGSGSVYGVV	6.0	6.6	2.2	3.0	0.3	
RP30NPB20-IGFR-G10	SFYSCLESLVNGGAERSDQWEGCRVGDHVRGEPLSLGVAQIM	12.5	6.4	1.2	5.5	0.2	
RP30NPB20-IGFR-C9	SFYSCLESLVNGGAERSDQWEGCRI SAATAQLGYGLPESGTRWR	7.8	6.3	1.4	4.6	0.2	
RP30NPB20-IGFR-B1	SFYSCLESLVNGGAERWEGCRQGGHFFEGRVGGANHHGV	5.5	6.3	2.4	2.6	0.4	
RP30NPB20-IGFR-B5	SFYSCLESLVNGGAERSDQWEGCRTAGVSSDGGSGVGFVVLGDNA	10.5	6.2	1.8	3.5	0.3	
RP30NPB20-IGFR-H9	SFYSCLESLVNGGAERSDQWEGCRWLAVAATGGGREADVGLRLS	14.0	6.1	1.2	5.1	0.2	
RP30NPB20-IGFR-H5	SFYSCLESLVNGGAERSDQWEGCRQSGVSSNSAGVRAQVSGMGG	8.0	6.0	1.5	3.9	0.2	
RP30NPB20-IGFR-C8	SFYSCLESLVNGGAERSDQWEGCRVGGVGRVTFPQGGFPRVVA	9.0	5.7	1.0	5.6	0.3	
RP30NPB20-IGFR-H7	SFYSCLESLVNGGAERSDQWEGCRFFESAGVQVQGLGTGRGSE	7.3	5.4	1.4	4.0	0.2	
RP30NPB20-IGFR-B9	SFYSCLESLVNGGAERSDQWEGCRLSWQGRVLVGGRDQTRGLQ	7.5	5.3	0.9	5.6	0.2	
RP30NPB20-IGFR-B11	SFYSCLESLVNGGERSDQWEGCRTITTEQRQRGGPDAGAGRS	9.2	5.2	1.3	3.8	0.3	
RP30NPB20-IGFR-H11	SFYSCLESLVNGGAERSDQWEGCRGAVGAPRGGVDVLSQSLR	7.4	5.2	1.7	3.1	0.3	
RP30NPB20-IGFR-D11	SFYSCLESLVNGGAERSDQWEGCRLRLIDRTGRQGVTPVHGPV	13.6	5.1	2.4	2.2	0.5	
RP30NPB20-IGFR-H4	SFYSCLESLVNGGAERSDQWEGCRTSLAIQVQDREAVAVGRGDL	9.2	3.4	1.1	3.1	0.3	
RP30NPB20-IGFR-B4	SFYSCLESLVNGGAERSDQWEGCRLQHPVLAKVPPGVRINFF	9.9	3.1	1.0	3.0	0.3	
RP30NPB20-IGFR-C12	SFYSCLESLVNGGAERSDQWEGCRDVPPLPLQWLGEPPWRVPGDNR	0.9	0.9	0.8	1.0	1.0	

FIG. 56B

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NPB20RP30 VS IGFR

Clone #	Sequence	E-Tag	IGFR	IR-Fc	IGFR/IR	IR/IGFR	SEQ ID NO:
Design	XXXXXXXXXXXXXXXXXXSFYSCLESILVNGGAERSDQWEGCR	10.9	4.3	1.3	3.5	0.3	
NPB20RP30-IGFR-H12	DGDVGGRVIARVGSFYSCLESILVNGGAERSDQWEGCR	10.6	10.4	1.9	5.5	0.2	
NPB20RP30-IGFR-H5	DGRQPRVAGGSANRVVAYGVSYSCLESILVNGGAERSDQWEGCR	13.8	2.7	1.4	2.0	0.5	
NPB20RP30-IGFR-F11	EDLVAAFGAGEGGHSFYSCLESILVNGGAERSDQWEGCR	10.8	3.0	1.0	2.9	0.3	
NPB20RP30-IGFR-A10	EGISMRLEVSFYSCLESILVNGGAERSDQWEGCR	15.5	9.4	2.0	4.7	0.2	
NPB20RP30-IGFR-D7	EKVIAIKASSGQKPLDPSSFYSCLESILVNGGAERSDQWEGCR	13.1	4.2	1.5	2.7	0.4	
NPB20RP30-IGFR-H9	ELGLRQPMVVGSGSPESGRSFYSCLESILVNGGAERSDQWEGCR	14.7	14.4	1.5	9.9	0.1	
NPB20RP30-IGFR-G8	FFGRSFYSCLESILVNGGAERSDQWEGCR	12.0	11.2	1.2	9.5	0.1	
NPB20RP30-IGFR-H8	FLKNAYGGPGKESDRARLVTSFYSCLESILVNGGAERSDQWEGCR	14.8	3.8	2.2	1.8	0.6	
NPB20RP30-IGFR-E10	GGATSSGGITSEFASNAGGLSFYSCLESILVNGGAERSDQWEGCR	4.3	1.3	1.0	1.3	0.8	
NPB20RP30-IGFR-A5	GGGPSTVQSVWGSFYSCLESILVNGGAERSDQWEGCR	15.2	13.1	2.3	5.6	0.2	
NPB20RP30-IGFR-F3	GGNGKTRSFYSCLESILVNGGAERSDQWEGCR	11.4	5.5	1.8	3.0	0.3	
NPB20RP30-IGFR-E4	GGSLYVDGRGGSGDEPDGAVSFYSCLESILVNGGAERSDQWEGCR	16.9	11.6	3.7	3.2	0.3	
NPB20RP30-IGFR-E7	GKILNDIPVTGRRNGFADNRFSFYSCLESILVNGGAERSDQWEGCR	15.6	14.9	5.6	2.7	0.4	
NPB20RP30-IGFR-D4	GLGGVTRSDVPVGLRRSNSISFYSCLESILVNGGAERSDQWEGCR	13.2	5.8	1.2	4.7	0.2	
NPB20RP30-IGFR-E8	GLIRWIADEVKASRVPTLTSSFYSCLESILVNGGAERSDQWEGCR	12.6	11.7	3.0	3.9	0.3	
NPB20RP30-IGFR-G10	GRASWKDGSNGSVPGSRVSYSCLESILVNGGAERSDQWEGCR	14.8	14.7	2.3	6.3	0.2	
NPB20RP30-IGFR-G9	GSNSSQGRVGLRGASDGVSYSCLESILVNGGAERSDQWEGCR	13.6	14.8	2.8	5.2	0.2	
NPB20RP30-IGFR-G3	HAGSLGMPGASEGRFTRLLSFYSCLESILVNGGAERSDQWEGCR	11.9	6.4	2.2	2.9	0.3	
NPB20RP30-IGFR-H7	HRAGPREEFYSGFLEIADGRSFYSCLESILVNGGAERSDQWEGCR	15.0	7.2	2.4	3.0	0.3	
NPB20RP30-IGFR-D5	LETFVGAGHAT.KINWRGPTSFYSCLESILVNGGAERSDQWEGCR	16.1	8.6	1.4	6.4	0.2	
NPB20RP30-IGFR-F7	LGQVRVGMVTRTVFSAGYVSFYSCLESILVNGGAERSDQWEGCR	11.1	9.2	1.7	5.4	0.2	
NPB20RP30-IGFR-H11	LPLGSH.GSPLGVIARV.GGSFYSCLESILVNGGAERSDQWEGCR	12.3	11.4	3.9	2.9	0.3	
NPB20RP30-IGFR-D3	LRTNPLHTF.GGVSGPGQSFYSCLESILVNGGAERSDQWEGCR	10.6	5.4	2.1	2.6	0.4	
NPB20RP30-IGFR-A3	LSTRYEYVSSVWSRVSSTHFSFYSCLESILVNGGAERSDQWEGCR	17.5	5.9	1.9	3.1	0.3	
NPB20RP30-IGFR-E5	NASLWSPGATDGDGRSNFKHSFYSCLESILVNGGAERSDQWEGCR	7.1	5.5	0.9	6.1	0.2	
NPB20RP30-IGFR-G11	QFDYHFGALGGTEIVDRQVSFYSCLESILVNGGAER.WEGCR	14.1	12.7	3.9	3.3	0.3	
NPB20RP30-IGFR-F10	QIVVAARGDVGGSFYSCLESILVNGGAERSDQWEGCR	7.8	8.1	2.1	3.9	0.3	
NPB20RP30-IGFR-H6	QLFSFSPDFTSGAIRGASFYSCLESILVNGGAERSDQWEGCR	9.3	9.8	3.8	2.6	0.4	
NPB20RP30-IGFR-A6	QVRVVGQPVGGFTRWEPGGNSFYSCLESILVNGGQWEGCR	11.9	11.4	2.2	5.1	0.2	
NPB20RP30-IGFR-H3	R.VAVISIGPESRRGRSEVRVSFYSCLESILVNGGAERSDQWEGCR	16.6	4.4	1.4	3.2	0.3	
NPB20RP30-IGFR-E6	RASNAAFIAMPISIRYAEAGSFYSCLESILVNGGAERSDQWEGCR	14.4	10.5	2.5	4.1	0.2	
NPB20RP30-IGFR-E3	RSFYSCLESILVNGGAERSDQWEGCR	14.7	16.0	5.2	3.1	0.3	
NPB20RP30-IGFR-D6	SDSPFELHMSPRDPWVRGVSFYSCLESILVNGGAERSDQWEGCR	10.2	7.4	1.8	4.1	0.2	
NPB20RP30-IGFR-E12	SGGAHALTE.FILFATPNRASFYSCLESILVNGGAERSDQWEGCR	14.7	3.0	1.6	1.9	0.5	
NPB20RP30-IGFR-F9	SHVSPAIPPTFNELDSQVMGISFYSCLESILVNGGAERSDQWEGCR	16.9	13.1	2.5	5.1	0.2	
NPB20RP30-IGFR-F5	SLRVKSDSTSMNPGTGLASISFYSCLESILVNGGAERSDQWEGCR	11.5	7.2	2.8	2.6	0.4	
NPB20RP30-IGFR-A11	SVFVGFQRQVSVGGPSFGHVFSFYSCLESILVNGGAERSDQWEGCR						

FIG. 57A

Clone #	Sequence	E-Tag	IGFR	IR-Fc	IGFR/IR	IR/IGFR	SEQ ID NO:
NPB20RP30-IGFR-A12	SVVDTRDGVGSLRRSAVSSSTSFYSCLESILVNGGAERSDGGWEGCR	13.7	6.9	1.8	3.8	0.3	
NPB20RP30-IGFR-F12	TGFAAMVTARRGSHAVTDDPSFYSCLESILVNGGAERSDGGWEGCR	9.5	9.7	2.4	4.0	0.2	
NPB20RP30-IGFR-G6	TQAGAHRSFTRLHKAMLQSSSFYSCLESILVNGGAERSDGGWEGCR	11.6	7.5	1.0	7.4	0.1	
NPB20RP30-IGFR-G5	TRWGV DSTILGHGRSILVRSFYSCLESILVNGGAERSDGGWEGCR	14.9	6.5	1.1	5.9	0.2	
NPB20RP30-IGFR-F6	VGPSRSGGWLGGIGDKSGVSFYSCLESILVNGGAERSDGGWEGCR	16.3	14.7	3.4	4.3	0.2	
NPB20RP30-IGFR-G12	VLRAENSRFSTASDFALSPVSFYSCLESILVNGGAERSDGGWEGCR	16.5	10.5	1.8	5.8	0.2	
NPB20RP30-IGFR-E11	WGGIEDRAFWALGGRSTTPSFYSCLESILVNGGAERSDGGWEGCR	16.7	14.4	2.4	5.9	0.2	
NPB20RP30-IGFR-F8	WLARGRSEVP SFYSCLESILVNGGAERSDGGWEGCR	12.5	14.0	4.3	3.3	0.3	
NPB20RP30-IGFR-H10	WNATMGSRNKSPGGGSAVEMSFYSCLESILVNGGAERSDGGWEGCR	9.7	12.2	6.1	2.0	0.5	
NPB20RP30-IGFR-E9	WOYDLIGSSRSRGDSRRSVSFYSCLESILVNGGAERSDGGWEGCR	16.7	13.4	5.7	2.3	0.4	
NPB20RP30-IGFR-H4	WSVATLHHVD . GAAPSPKVL SFYSCLESILVNGGAERSDGGWEGCR	14.3	7.8	2.3	3.4	0.3	
NPB20RP30-IGFR-D9	WVGGRAGSVRLYAVDGVVGGSFYSCLESILVNGGAERSDRQWEGCR	15.1	10.0	2.5	4.0	0.3	

FIG. 57B

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D815 vs IGFR	Sequence	ETAG	IGFR	IR	IGFR/IR	SEQ ID NO:
Clone	<u>WLDQEWAWVQCEVYGRGCP</u> <u>S</u>	--	--	--	--	--
Design	<u>GLDQDRAWLWCEISGHGCL</u> <u>S</u>	36.8	5.7	1.0	5.7	
D815-IGFR-A10	<u>GVDQERAWLWCISGRGC</u> <u>Q</u> <u>S</u>	17.6	5.6	0.5	12.0	
D815-IGFR-A11	<u>GLAEERAWLWCISGRGC</u> <u>L</u> <u>S</u>	37.1	16.6	1.3	12.7	
D815-IGFR-A12	<u>GLEEERAWLWCISGRGC</u> <u>L</u> <u>S</u>	38.0	15.3	0.9	16.3	
D815-IGFR-A2	<u>GLDEERAWLWCISGRGC</u> <u>L</u> <u>S</u>	39.6	7.2	1.0	7.3	
D815-IGFR-A3	<u>SLEQERAWLWCISGRGC</u> <u>S</u> <u>S</u>	39.8	9.4	1.1	8.8	
D815-IGFR-A4	<u>SLDQERAWLWCISGRGC</u> <u>L</u> <u>S</u>	42.5	10.5	1.0	10.2	
D815-IGFR-A5	<u>WLDQERAWLWCEISGLGC</u> <u>P</u> <u>S</u>	35.1	4.2	0.9	4.7	
D815-IGFR-A6	<u>QPDQVRAWLWCEISGRGC</u> <u>L</u> <u>S</u>	36.5	10.3	1.2	8.8	
D815-IGFR-A8 ₃	<u>QLDEERAWLWCEISGLGC</u> <u>L</u> <u>R</u>	34.1	2.2	1.1	2.0	
D815-IGFR-A9	<u>GLDQERAWLWCEISGHGC</u> <u>L</u> <u>P</u>	31.8	3.5	0.9	3.9	
D815-IGFR-B1	<u>WLDQERAWLWCEISGGC</u> <u>L</u> <u>S</u>	30.9	5.7	1.2	4.9	
D815-IGFR-B3	<u>GLDEERAWLWCISGRGC</u> <u>L</u> <u>S</u>	31.6	12.5	0.9	13.7	
D815-IGFR-B4	<u>WLDKERAWLWCEISGHGC</u> <u>L</u> <u>S</u>	29.8	2.0	0.9	2.2	
D815-IGFR-B5	<u>QLAQERAWLWCISGRGC</u> <u>L</u> <u>S</u>	31.0	2.9	0.9	3.1	
D815-IGFR-B6	<u>GLDEERAWLWCISGGC</u> <u>V</u> <u>P</u>	30.4	2.4	0.9	2.7	
D815-IGFR-B8	<u>WLDREERAWLWCEISGLGC</u> <u>L</u> <u>S</u>	30.3	2.4	1.0	2.4	
D815-IGFR-C10	<u>QGDQVRAWLWCISGRGC</u> <u>L</u> <u>S</u>	22.7	4.4	0.7	6.0	
D815-IGFR-C12	<u>RLDQERAWLWCISGRGC</u> <u>L</u> <u>S</u>	31.4	2.8	0.9	3.2	
D815-IGFR-C3 ₂	<u>GPDQVRAWLWCEISGRGC</u> <u>L</u> <u>S</u>	32.3	9.9	1.0	9.9	
D815-IGFR-C4	<u>GLDQDRAWLWCEISGRGC</u> <u>R</u> <u>S</u>	32.5	4.2	1.0	4.4	
D815-IGFR-C5	<u>WLDREERAWLWCEISGYGC</u> <u>L</u> <u>S</u>	25.9	1.5	0.8	1.9	
D815-IGFR-C6	<u>WLDREERAWLWCEISGRGC</u> <u>L</u> <u>S</u>	26.6	2.5	0.8	3.1	
D815-IGFR-C7	<u>GLAEDRAWLWCEISGRGC</u> <u>V</u> <u>S</u>	32.6	12.4	0.9	13.8	
D815-IGFR-C8	<u>QVDEVRAWLWCEISGRGC</u> <u>L</u> <u>S</u>	32.6	3.8	1.0	3.8	
D815-IGFR-D10	<u>GVEQERAWLWCEISGLGC</u> <u>L</u> <u>S</u>	29.5	10.7	0.9	11.5	
D815-IGFR-D11	<u>GPEQDRAWLWCISGRGC</u> <u>L</u> <u>S</u>	26.5	4.0	0.9	4.7	
D815-IGFR-D12	<u>SLDQERAWLWCISGRGC</u> <u>L</u> <u>S</u>	25.3	4.7	0.8	5.8	
D815-IGFR-D1	<u>GLEEERAWLWCEISGLGC</u> <u>L</u> <u>S</u>	31.6	17.1	0.9	18.5	
D815-IGFR-D5	<u>SLDEDRAWLWCISGRGC</u> <u>L</u> <u>S</u>	33.3	14.6	0.9	16.1	
D815-IGFR-D6	<u>WLDREERAWLWCEISGLGC</u> <u>P</u> <u>S</u>	24.6	3.0	0.8	3.9	
D815-IGFR-D7	<u>WLDREERAWLWCEISGLGC</u> <u>E</u> <u>S</u>	23.1	4.2	0.8	5.2	

FIG. 58A

Clone	Sequence	ETAG	IGFR	IR	IGFR/IR	SEQ ID NO:
D815-IGFR-D8	WLDEERAWLWCEISGHGCLS	30.2	2.7	1.0	2.7	
D815-IGFR-D9	RVDQVRAWLWCEISGLGCPs	29.3	12.5	1.0	13.0	
D815-IGFR-E12	GLDEERAWLWCQITGRGCLS	26.8	7.1	0.7	9.8	
D815-IGFR-E1	WLDQERAWLWCEISGLGCLS	27.2	2.9	0.8	3.5	
D815-IGFR-E2	SLDQERAWLWCQISGRGCLT	33.2	6.9	1.0	7.1	
D815-IGFR-E3	GLDQTRAWLWCQISGRGCLF	24.9	2.8	0.8	3.7	
D815-IGFR-E4	GPDELRAWLWCEISGRGCLS	33.6	18.1	1.0	17.5	
D815-IGFR-E6	QLDQVRAWLWCEISGQGRS	20.8	3.0	0.9	3.5	
D815-IGFR-E9	WLDRERAWLWCEISGLGCLA	23.9	1.9	0.9	2.1	
D815-IGFR-F10	GHDQVRAWLWCQISGSGCLS	11.7	2.4	0.4	6.5	
D815-IGFR-F11	WLDRERAWLWCEISGEGCLS	25.6	4.2	0.8	5.5	
D815-IGFR-F5	GLDQDRAWLWCQISGRGCLS	32.2	7.7	1.0	7.7	
D815-IGFR-G10	GLDQERAWLWCQISGRGCMs	32.0	5.2	1.0	5.3	
D815-IGFR-G1	QLDRDRAWLWCEISGLGCLS	31.1	3.6	1.0	3.8	
D815-IGFR-G2	GVDEERAWLWCQISGLGCMF	33.1	14.0	1.0	13.8	
D815-IGFR-G3	SLEQERAWLWCEISGIGCMS	30.3	11.8	1.0	12.2	
D815-IGFR-G4	GLDQERAWLWCQISGRGCLS	32.3	6.7	1.0	6.6	
D815-IGFR-G5	WLDEERAWLWCEISGQGCLS	31.2	6.2	1.0	6.0	
D815-IGFR-G6 ₃	SPEQIRAWLWCEISGIGCVs	28.6	15.9	0.9	16.8	
D815-IGFR-G7	LLDQERAWLWCEISGQCCQS	26.3	2.6	1.0	2.5	
D815-IGFR-G8	GLEQERAWLWCQISGRGCLS	31.7	6.2	1.0	6.2	
D815-IGFR-H2 ₃	WLDRERAWLWCEISGLGCLS	24.3	2.4	0.7	3.2	
D815-IGFR-H4	WLDQERAWLWCEISGQGCLS	32.0	4.8	0.9	5.3	
D815-IGFR-H7	SLDQERAWLWCEISGLGCVs	30.1	6.0	0.9	6.3	

FIG. 58B

RP6-D815 VS IGFR

Clone #	Sequence	E-Tag	IGFR	LDH	Sp/Ir	SEQ ID NO:
Design		--	--	--	--	
RP6-D815-IGFR-4-D7	TFYSCLASLLTGTPQPNRGWERCRLDQERAWLWCEISGRGCLS	20.0	18.3	1.1	17.1	129/163
RP6-D815-IGFR-4-B11	GSQPERGLRERSRLDQERAWLWCEISGRGCLS	17.6	17.6	1.0	16.9	
RP6-D815-IGFR-4-C9	SFYSCLASLVGTSSQPKRGWGLS	19.5	15.9	1.0	16.2	
RP6-D815-IGFR-4-G11	SRLFGSAQLNGGAGERSRLDQERAWLWCEISGRGCLP	16.4	15.7	1.0	16.4	
RP6-D815-IGFR-4-F7	WERSPWLEQERAWLWCEISGRGCVS	20.8	14.5	1.2	12.1	
RP6-D815-IGFR-4-E9	SPRERGRSLDLDRAWLWCVISGRDCGA	17.3	13.0	1.0	13.2	
RP6-D815-IGFR-4-F2	SFYCGLAALLTGTPADRPWDASGEISGRGCLS	16.9	12.3	1.1	11.2	
RP6-D815-IGFR-4-H6	TLTSLDSLSQGTQPSRGPSERRRWLEQERAWLWCEISGGGCLS	14.5	11.8	1.1	11.2	
RP6-D815-IGFR-4-F9	SFYDCLATLLTGTPQPTRGSGRIGERGCRS	15.5	11.1	1.0	10.9	
RP6-D815-IGFR-4-G5	SERSRWLLQERAWLWCEISGRGCV	9.2	10.2	1.1	9.1	
RP6-D815-IGFR-4-F10	SFYSCLDALLNGRKPNAGELCSWRAQERARPAEICGRCRT	18.0	10.0	1.0	10.4	
RP6-D815-IGFR-4-H8	TERRLWLDREAWLWCEISGRGCLS	16.6	9.9	1.1	8.8	
RP6-D815-IGFR-4-C4	SFYSCLASLLTGTPDENRGWFRCLDQAGDLWGEPSGRGLVS	19.3	9.7	1.1	8.9	
RP6-D815-IGFR-4-D11	TERRLWLDREAWLWCEISGRGCLS	19.6	9.6	1.0	9.6	
RP6-D815-IGFR-4-A9	SFYSGGWERRSWLDQERAWLWCEISGRGCPA	14.1	8.8	1.0	8.8	
RP6-D815-IGFR-4-H11	SFYSCLGALLAGPPQPGRLWRCRWRDEGGAWQCENSGRACLS	6.4	8.4	1.0	8.7	
RP6-D815-IGFR-4-F1	HSEIAVASLRSRQLEQERAWLWCEISGRGCLA	11.5	8.1	1.1	7.1	
RP6-D815-IGFR-4-G12	SFYSCLTSLSGADGGPRRCRW	13.1	7.9	0.9	8.6	
RP6-D815-IGFR-4-G9	RSYSSLDSEGGTAQPNRGTLERGGWLDQERAWLWCEISGRGCLA	14.0	7.8	1.4	5.5	
RP6-D815-IGFR-4-F12	HVPLVSVGDGARDPIRGWPQRIHWLDEDDRAWLWCEISGRGCRS	15.0	7.4	0.9	8.2	
RP6-D815-IGFR-4-G2	SFYSCLSLVGTGAQREPSWERCRLDQERAWLGGGLAGRGGLS	15.6	7.2	1.5	4.9	
RP6-D815-IGFR-4-D8	SGYSDRWRLDQDRAWLWCEISGRGCLA	20.1	7.1	1.1	6.4	
RP6-D815-IGFR-4-G8	TFYSCLDLTLTATPRRQDGVGLWSEISGRGCRS	18.0	7.1	1.1	6.5	
RP6-D815-IGFR-4-B7	NFYSCLASLLSATPPQPRGGGCLS	19.3	6.9	1.0	6.7	
RP6-D815-IGFR-4-H9	TFYSCLASLLADKAQPNRSGEGWREIAGRECLS	16.7	6.9	1.6	4.3	
RP6-D815-IGFR-4-B3	RGSSDRSLWLDQDRAWLWCEISGRGCLS	19.0	6.8	1.0	6.5	
RP6-D815-IGFR-4-A2	SFYSCLASLVGTGAQSNRGSWERCRRGLY	17.3	6.7	9.5	0.7	
RP6-D815-IGFR-4-F3	TLYSGQESMGSGSPQLDRGPGRVRLDQVRAWLWCEISGHGCLS	14.2	6.7	0.8	8.3	
RP6-D815-IGFR-4-C8	SPRERGRSLDLDRAWLWCVISGRDCGA	20.0	6.5	0.9	7.1	
RP6-D815-IGFR-4-D1	RGPSEHSRWLERERAWLWCEMSGRGCVS	10.9	6.5	1.0	6.4	

FIG. 59A

Clone #	Sequence	E-Tag	IGFR	LDH	Sp/Ir	SEQ ID NO:
RP6-D815-IGFR-4-F4	SFYSCLASLMTGEISANGCLS	9.5	6.4	1.0	6.6	
RP6-D815-IGFR-4-C2	SFYSCLASLLTATSQPNRGPDRCSGGWLS	11.1	6.1	3.7	1.6	
RP6-D815-IGFR-4-H1	KIQAGPASLLTGITQPNGGAWERYRSLDQERAWLWCQISGRGCE	8.8	5.3	5.9	0.9	
RP6-D815-IGFR-4-B2	TFASGRAASLNGSPKSDGGPWGRGSSLDEERAWLWCEISGRGCLH	18.6	4.8	0.9	5.6	
RP6-D815-IGFR-4-C3	TFYSCLASLLTGSAGCGRGEGRGLEKERACVWGDMAGRGCQS	10.9	4.7	1.0	4.6	
RP6-D815-IGFR-4-A4	SFYSCMASLLNGPRERNPGQRECFRGREPGRASRGCELSGRGCVT	13.8	4.3	0.9	4.7	
RP6-D815-IGFR-4-E8	TFYSCLASLLADKAQPNRSGEGWREIAGRECLS	8.7	4.2	1.0	4.3	
RP6-D815-IGFR-4-E1	SFYSCLAFLLRGTPQPCNPGPGERCRWSDQDRAQLWCEQPGTGSL	12.7	4.1	1.2	3.3	
RP6-D815-IGFR-4-A10	TCYSQVAPLLNGTLLPSRGQGERSRWLDQQRAWLWCEISGRGCLT	5.0	4.0	1.2	3.3	
RP6-D815-IGFR-4-A11	SLCSGMACVLNGTAQPNRGLLKRRWLDQERAWLWCEISGRGCQS	3.4	3.6	0.9	3.8	
RP6-D815-IGFR-4-H12	TRNNGLRWVDQERAWLWCEISGRGCLF	8.8	3.1	0.8	4.0	
RP6-D815-IGFR-4-H7	SPRERGRSLDLDRAWLWCVISGRDCGA	6.5	3.1	1.2	2.6	
RP6-D815-IGFR-4-B6	SFEQWVASLRAGTPQPQGGPSGRIRSVDDQERAWLWCEISGRGCLS	15.9	2.8	1.1	2.5	
RP6-D815-IGFR-4-E10	TSYSQQASLPTGTATQPNRRPSPERWRWLDQERAWLWCEISGRGCR	4.1	2.0	1.0	2.1	

FIG. 59B

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RP6-6-D815 vs IGFR

CLONE#	Sequence	Etag	IGFR	LDH	IGFR/LDH SEQ ID NO:
Design	TFYSCIASLLTGTPQPNRGPGSGSWLDQERAWLWCEISGRGCLS	--	--	--	--
RP6-6-D815-IGFR-4-H10	SLSAYRGGSGGGDRERAWLWCEISGHGCVS	14.9	17.7	1.3	14.1
RP6-6-D815-IGFR-4-D11	SFYSCIAHLMGTPPQPKGGPWERYCGQS	12.7	17.4	1.4	12.8
RP6-6-D815-IGFR-4-B12	QLDQCFYSCLEYLRTGAPQTDGGPWERC	11.7	17.4	1.3	13.7
RP6-6-D815-IGFR-4-D9	SFESYLGPLLTSTTQPNRDRGRGGSGSWLEQERAWLWCEIAGRGCLS	13.4	17.3	1.6	10.7
RP6-6-D815-IGFR-4-C9	STVSRGGSGSWLDQQRRAWLWCEISGHGCLS	12.8	17.2	1.4	12.6
RP6-6-D815-IGFR-4-A2	SFYSGIACLLSGPAQPNRGKCCERSGDLP	12.7	17.1	1.8	9.4
RP6-6-D815-IGFR-4-B11	SFESCLAWLLTSRPLSDSVWDRGRGRYS	12.8	17.1	1.2	14.3
RP6-6-D815-IGFR-4-D1	SFYSCIASLLSGTPQQTGGRWGRGCLS	12.5	17.0	1.4	12.4
RP6-6-D815-IGFR-4-C8	RIGSGSGSWLDQERAWLWCKISGRGCLS	12.7	16.2	1.5	10.6
RP6-6-D815-IGFR-4-A11	SFYSCIASLLTGAPERDNLVCEMSSRWGWS	11.6	16.0	1.1	15.1
RP6-6-D815-IGFR-4-E7	RSFYDCLTFVLNGPPQPNGRWERC	13.0	16.0	1.5	10.7
RP6-6-D815-IGFR-4-B9	TFYSCLDLSMSGTPQSLRGRWERCYGA	11.5	15.8	6.9	2.3
RP6-6-D815-IGFR-4-D5	GLSTRGSQLAQERAWLWCEISGRGCVS	12.4	15.8	1.2	13.1
RP6-6-D815-IGFR-4-C6	SGSGSGSWDRQRAWLWCEISGRGCLS	13.2	15.7	1.5	10.4
RP6-6-D815-IGFR-4-E12	WERRCGSGSWLDQQRRAWLWCEISGRGCVS	10.6	15.6	1.0	16.0
RP6-6-D815-IGFR-4-B5	TLYACAASRPTGTPQPNLGSWAGSRGGSRLDQERAWLWCEISGLGCM	12.9	15.6	1.2	12.8
RP6-6-D815-IGFR-4-B4	SFYSCIASLVTGNPQNRGCLS	11.6	15.6	1.3	12.3
RP6-6-D815-IGFR-4-H12	SFYTCLSSLLTGTPPNRGPWELSRGCVT	12.1	15.5	1.1	14.2
RP6-6-D815-IGFR-4-B6	TFYSCIASLLDGTTPHRCGWERCGRGCLS	12.1	15.4	1.4	11.2
RP6-6-D815-IGFR-4-D7	SFYSCIASLVTGAGKSPGRGCLG	11.7	15.4	1.2	13.2
RP6-6-D815-IGFR-4-D10	SFYSSMACLLNGTLPDRGRWRACLT	11.2	15.3	1.5	10.6
RP6-6-D815-IGFR-4-B7	SFYSCLDTLGGTPQQRNSPWRDRCRGGVS	11.8	15.3	5.9	2.6
RP6-6-D815-IGFR-4-E10	HSRGGSGSWMEKERAWLWCEISGCGSS	14.2	15.2	1.6	9.7
RP6-6-D815-IGFR-4-C3	SFYSCLDTLTGASWKRCRGCLS	12.0	15.0	1.3	11.2
RP6-6-D815-IGFR-4-F10	TFYSCLDLSLLTGTPPEPNRGWERCGRGGY	12.6	14.9	1.4	10.4
RP6-6-D815-IGFR-4-E6	SFYSCLDTLTGASWKRCRGCLS	11.8	14.9	1.4	10.5
RP6-6-D815-IGFR-4-F7	TFYSCIASLVTGAPGPKRGWERCRRGLP	11.1	14.7	1.5	9.5
RP6-6-D815-IGFR-4-A5	NQTVVDGYVVVGGSGSWLDQERAWLWCEISGRDCPT	11.5	14.6	1.2	12.7
RP6-6-D815-IGFR-4-C10	SFYSCLDTLTGASWKRCRGCLS	11.2	14.5	1.5	9.5
RP6-6-D815-IGFR-4-A7	SFYSCIASLLTGTPQTNRGAWDRCRGGQS	11.1	14.5	1.0	14.5

FIG. 60A

CLONE#	Sequence	Etag	IGFR	LDH	IGFR/LDH SEQ ID NO:
RP6-6-D815-IGFR-4-F9	DFYSGLACTINGSPEEKHPWERCGRGGL	12.0	14.4	1.3	11.1
RP6-6-D815-IGFR-4-B8	SFYSCDLTLLTGASWKRGRGGLS	12.2	14.3	1.4	10.2
RP6-6-D815-IGFR-4-F12	TFYSCLASLLTDIPEQNRGPRDRCRGGLA	10.1	14.2	0.9	15.7
RP6-6-D815-IGFR-4-A10	TFYSCLASLLSGPPPNLDPWDRCRGPPS	10.4	14.2	1.3	10.9
RP6-6-D815-IGFR-4-E3	SFQSCLASLLTGTTLPGGTWERCGRWPS	10.9	14.2	1.2	11.9
RP6-6-D815-IGFR-4-C4	SFYSCDLTLLTGASWKRGRGGLS	11.3	14.1	1.3	11.0
RP6-6-D815-IGFR-4-C7	TSSLLPNRGAQIRIGSGGSLAQERAWLWCEITGRGCLS	12.8	14.0	1.4	10.3
RP6-6-D815-IGFR-4-C5	TFYSCLASLLNGTTPHNRGPWEISGRGCLS	10.5	14.0	1.7	8.0
RP6-6-D815-IGFR-4-A12	SFHSCLASLVNGTWRPNRGVCEISGRGVQ	10.0	14.0	0.9	15.2
RP6-6-D815-IGFR-4-C11	SFYDCLASLLSVTSLPNRPQDRCRGFLS	11.3	13.7	1.4	10.1
RP6-6-D815-IGFR-4-F11	TFYSCLDQLETTGTPNARGAWERCGRGLF	11.2	13.6	0.9	15.9
RP6-6-D815-IGFR-4-F6	GSVRGNVGGGSLDRQRAWLWCEISGRGCPs	10.7	13.5	1.4	10.0
RP6-6-D815-IGFR-4-C2	VRPDRGTCERCRRGGGSLDQERAWLWCEISGRGCLP	10.8	13.4	1.3	10.0
RP6-6-D815-IGFR-4-D6	SVSKWLERERARLWCEISGLGCLS	11.7	13.3	1.1	11.9
RP6-6-D815-IGFR-4-E1	SFYSSMACLLNGTTPDRGPRERWRACLT	11.7	13.3	1.7	8.1
RP6-6-D815-IGFR-4-A9	TVGNVGGSLRDQVRSWLWCEISGRGCMs	12.0	13.1	1.3	9.9
RP6-6-D815-IGFR-4-G11	NFQSCLASLVGTALPNRGTWERCGRFPA	11.1	13.1	1.0	13.2
RP6-6-D815-IGFR-4-D2	TFYSCLASLLSGPPKPNRDPQESCRGWLs	9.2	13.0	1.3	10.3
RP6-6-D815-IGFR-4-A3	SFYSCDLTLLTGASWKRGRGGLS	7.9	13.0	1.2	10.6
RP6-6-D815-IGFR-4-E9	SFYSCLAALLTGVPQPNVGPWERCGRPRY	11.1	13.0	1.5	8.9
RP6-6-D815-IGFR-4-E11	SFYSCLSLLNGNLPRNPGRWEGCRGGLs	11.9	12.9	1.3	10.2
RP6-6-D815-IGFR-4-A8	NMVGGQDRGGGSGGSLDKERAWLWCEISGLGCRs	10.5	12.8	1.3	9.9
RP6-6-D815-IGFR-4-H11	TLGGSGGSLDRERAWLWCEMTGRCRS	10.7	12.7	1.1	11.2
RP6-6-D815-IGFR-4-E5	TSYSCLASLLPDSPQPNRGQRRRGSGGSLD.ERAWLWCEISGRGCLS	8.0	12.6	1.2	10.2
RP6-6-D815-IGFR-4-F5	TFYSCLTSLMSGGTWEHCRGGGSLDQERAQQCRGITGRGCPs	8.9	12.6	1.0	12.8
RP6-6-D815-IGFR-4-D4	TFYSCLASLLTGTPLHNRGPWERCGRDGLs	10.7	12.5	1.1	11.5
RP6-6-D815-IGFR-4-G8	NSASWIDQERAWLWCEISGRGCLS	9.7	12.3	1.1	11.1
RP6-6-D815-IGFR-4-G9	TFYSCDLFLLAGTTPARGPWEHCRGGMs	10.4	12.2	1.5	8.0
RP6-6-D815-IGFR-4-B1	SFYSCLTSLTGTTPKPNRGCVs	7.5	11.9	1.3	9.5
RP6-6-D815-IGFR-4-F3	TFYSCLDLSLTSTAQVKPGPWERCGRSMS	9.7	11.8	1.4	8.6
RP6-6-D815-IGFR-4-G5	SFYSCLASLLPGTALPNRGCRs	9.4	11.3	1.0	11.1
RP6-6-D815-IGFR-4-B2	GFYDCLASLVTPPESKRGAWERCGRSGS	11.2	11.2	1.5	7.6
RP6-6-D815-IGFR-4-A4	TFYDCLASLLTDTGQSRGRWERCGRGGLs	9.7	11.1	1.1	9.7
RP6-6-D815-IGFR-4-G10	TFYSCLAYLVGTTPKPYRGPGETWRCCLs	10.2	11.0	1.4	7.9
RP6-6-D815-IGFR-4-D3	SFYSCLSALLAGSPENRGPQRCRGSLL	10.1	11.0	1.1	10.2

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FIG. 60B

CLONE#	Sequence	Etag	IGFR	LDH	IGFR/LDH SEQ ID NO:
RP6-6-D815-IGFR-4-E2	TFYSCLASLVSGSPQRSGPWERCRRGGQS	8.8	10.9	1.3	8.4
RP6-6-D815-IGFR-4-H8	PAMEDRWYVGRGGSGSWLDQERAWLWCEISGRGCLS	8.3	10.9	1.4	7.6
RP6-6-D815-IGFR-4-E4	TFYSCLDDELVTGTPTQLTRGPWERCGRWLS	8.1	10.8	1.1	9.7
RP6-6-D815-IGFR-4-F1	GFYDCLASLVGTGPPESKRGAWERCRRGSGS	9.0	10.5	1.2	8.5
RP6-6-D815-IGFR-4-H7	SFSSAGSLLANDSQPSAVRGTLRGDSGGSWLDQERAWLWCEISGRGCLS	10.3	10.4	1.8	5.9
RP6-6-D815-IGFR-4-F4	NFYTGTLPLLSDRTEQNRRGMDGRRGGSGSWLDQERAWLWCEISGRGCLS	7.9	10.3	1.0	10.6
RP6-6-D815-IGFR-4-H9	SKPNRDTWERCRRGGSGSWLDQERAWLWCEISGRGCLS	8.0	10.1	1.2	8.5
RP6-6-D815-IGFR-4-G1	SFYSSMACLLNGTLPDRGPRWRACLT	5.7	10.1	1.0	10.5
RP6-6-D815-IGFR-4-A6	SFYSCLAALLVGNPERERCPWVRCRGGLY	12.8	10.1	1.2	8.7
RP6-6-D815-IGFR-4-G2	TFYSCLASLRTAPPPNRGPWEGCRGGLS	7.2	9.6	1.0	9.3
RP6-6-D815-IGFR-4-G3	SFYSCLVGLMNATPDPSRGVWQSCRRGPP	7.4	9.3	0.8	11.6
RP6-6-D815-IGFR-4-G12	SFYSCLASLVGTGTPQACRGPWERCRRGGLS	8.9	9.0	1.0	9.3
RP6-6-D815-IGFR-4-G4	RSWLDKERAWLWCEISGGGCLT	6.7	8.7	0.8	11.6
RP6-6-D815-IGFR-4-H3	SFYSSMACLLNGTLPDRGPRWRACLT	6.4	8.5	0.8	11.0
RP6-6-D815-IGFR-4-G6	VRGGSRLDLERAWLWCEISGRGCLS	9.6	8.4	1.2	7.1
RP6-6-D815-IGFR-4-H1	TCFSCLGSRAGTPERNRSGMGGSGSWLGRERAWLWCEISGRGCLS	5.9	7.6	1.0	7.8
RP6-6-D815-IGFR-4-H6	SSGERRGSGSDSWLGEVRAWLWCEISGGCQS	6.2	7.5	1.3	5.7
RP6-6-D815-IGFR-4-H2	SFYSCLDTLTLTGASWKRCRRGGLS	6.6	7.5	0.9	8.8
RP6-6-D815-IGFR-4-F2	TFYACLANLLSGTPEASRGTWERCRRGRES	6.2	7.4	1.1	6.8
RP6-6-D815-IGFR-4-A1	TFYDCLASLLTQPNRGRGRL	7.8	6.5	1.5	4.3
RP6-6-D815-IGFR-4-H4	TFYSCLASLVGTGTPQDRGWERCRGGFF	5.6	6.0	0.8	7.2

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FIG. 60C

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RP6-0-RP9 vs IGFR	Clone #	SEQUENCE	E-Tag	IGFR	LDH	Sp/Irr	SEQ ID NO:
Design		TFYSCLASLLTGTPQPNRGPWERCGRGSLDESYDWFERQLG	--	--	--	--	--
RP6-0-RP9-IGFR-B11		TLNPRGPWEGSRGSMDDSYFWFERQLE	27.6	25.7	1	25.2	1
RP6-0-RP9-IGFR-H2		TGAPQPNRGPLDRRCRGSDECFYGWFERQLL	16.6	21.1	1	20.7	1
RP6-0-RP9-IGFR-E9		RGNVGGSLDESIFYEWFERQLG	19.8	20.5	0.8	24.2	0.8
RP6-0-RP9-IGFR-C1		TFYSGPVSLLTGTPRTNRSAWERCGRGSLDDSYDWFERQLS	19.6	18.3	0.7	27.7	0.7
RP6-0-RP9-IGFR-D2		TMYSLVGFLPSGVGRPDRGPWERCGRGALDESYSWFERQLD	21.8	17	0.8	22.6	0.8
RP6-0-RP9-IGFR-E1		SLYSCMAALPNGTPQPKPGSWDRSGGHQDDSYDWFERQLG	20.2	16.4	0.9	18.5	0.9
RP6-0-RP9-IGFR-C5		SLYSGLAFLSGSPENRGRWARGRGRSDESYFGWFERQLA	21.3	15.5	0.8	18.5	0.8
RP6-0-RP9-IGFR-C4		TLHAGLDSLVTGPQERNRGPWERSRGALDEAFYGWFERQLG	17.8	15.3	0.9	17.5	0.9
RP6-0-RP9-IGFR-G4		MSSCLASLLTRTPEPNRGRWASGRGPLDGSFYDWFERQLG	21.3	15.2	0.9	16.1	0.9
RP6-0-RP9-IGFR-E7		SLTSLAAMLPGSQQTGGLALVSGSLDVSYEWFERQLG	18.5	13.6	0.9	14.9	0.9
RP6-0-RP9-IGFR-F9		TFYSRLVSLPTGTQQPNSGRWLSSSFVDESIFYGWFERQLG	18.2	13.4	0.9	15.2	0.9
RP6-0-RP9-IGFR-B7		TYYSGRGSLDESIFYDWFERQLG	20.2	13.1	0.9	15.3	0.9
RP6-0-RP9-IGFR-C3		SFYSCLSQLLIGIPHNRPDPWESCRGSLDEPFFEWFERQLG	16	11.5	0.7	17.2	0.7
RP6-0-RP9-IGFR-H3		SCLSCPASLLTGTPQANRGPWRRCRGSDECFYEWFDRLG	15.5	11.5	0.8	14	0.8
RP6-0-RP9-IGFR-H10		TIYSWQAAPQTGTPQLHRVPQESCRGSLDECFYDWFERQLG	10.3	11.4	1.1	10.8	1.1
RP6-0-RP9-IGFR-E5		SFQTHLASLVTGTAVTNHWGWERGSGSLDGSFYDWFERQLG	17.2	10.9	0.7	15.1	0.7
RP6-0-RP9-IGFR-A7		NLYSWVPSVRTGTPQPNRGAWECAGSLDIGFYEFHQHGLG	21.8	10.6	0.8	12.6	0.8
RP6-0-RP9-IGFR-G9		TVYCCVASLVTGTPQASGGAWERGRGSLDEAFYDWFERQLG	11	10	1.1	9.2	1.1
RP6-0-RP9-IGFR-A2		TFDNCIASLVTGSPETRRGPWERSRGSVDEYFYDWFERQVA	15	9.9	0.6	17.2	0.6
RP6-0-RP9-IGFR-E3		TSSGSASLVTDSQPKRGQVRCGGSVNECFYAWFERQLG	13.6	9.8	0.9	11.1	0.9
RP6-0-RP9-IGFR-B10		SLYSCGASHHNGTLQPNGGPSDRACAGSLDESIFYAWFERQLG	13.8	9.4	0.8	11.4	0.8
RP6-0-RP9-IGFR-H4		SSLSCLAALQNVAQASRAGERCGLGSRDECFYAWFERQLA	10.8	9.3	1.1	8.5	1.1
RP6-0-RP9-IGFR-D11		TFHGCCLASMTVAPVPNRGPWGRGRGSEDESIFYDWFEOQLG	15.3	8	0.8	9.6	0.8
RP6-0-RP9-IGFR-G11		QMRWFSEESFYDWFDRQLS	19.4	7.8	1.1	7.1	1.1
RP6-0-RP9-IGFR-F8		TFHPWLARLLTGTPQQNGGAWDPSRGSLDEQFYGWFERQLG	7	7.4	0.9	8.2	0.9
RP6-0-RP9-IGFR-H8		TSYSRLASMLTGTSQPNRGAWDRGRGSVDEAFYDWFERQLD	7.5	7.3	1.2	6.1	1.2
RP6-0-RP9-IGFR-D5		SFYSCLASLLAGGPETNRGPWRCRLSPESIEDWGSQRG	12.8	7.1	0.9	8.2	0.9
RP6-0-RP9-IGFR-A9		TFQAYLVSFQTGNPQTNRGPLEGGRGSLDQCFYDWFEPQVG	10.8	7	0.9	7.5	0.9
RP6-0-RP9-IGFR-H5		TFYSSLASLLTVAPQASLGPQGRGSLDESIFYEWFERQLG	10.2	6.7	1.1	5.9	1.1
RP6-0-RP9-IGFR-F7		TFDAQLASLTPRPPHANAGPRRCRGSDECFYDWFERQLG	7.6	6.6	0.9	7.3	0.9
RP6-0-RP9-IGFR-F10		TFYSSLSGLLTGSPQPNRGSNERGRGPLDEAFYEWFERQLG	4.2	6.1	0.8	7.6	0.8
RP6-0-RP9-IGFR-E10		NSYSFLAAHLIGSPPRNGGWERGRGSLDESIFYEYFERMLG	13.9	6	0.8	7.8	0.8
RP6-0-RP9-IGFR-A3		SLYSCPDYRLTGAPRPNGGQWGRGRGPLDESIFYGWFERGLG	10.4	5.6	0.5	10.7	0.5

FIG. 61A

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Clone #	SEQUENCE	E-Tag	IGFR	LDH	Sp/Irr	SEQ ID NO:
RP6-0-RP9-IGFR-C3	TVYSWLGVLQTNPHPNRDPWEGRGSLDESFYDWFERQLG	9.3	5.6	0.9	6.3	
RP6-0-RP9-IGFR-B4	SFYDCLASLLTGPQPNRGSDLCRGSLDETFKDWDEHRLG	4	5.1	0.8	6.3	
RP6-0-RP9-IGFR-C9	SFYSCIASLLTGTGPQPNRGSDRCRGSLDESNDQDWFPRG	8.6	5.1	0.9	5.9	
RP6-0-RP9-IGFR-G6	ESFYDWFPRQLG	13.7	5.1	0.9	5.9	
RP6-0-RP9-IGFR-A8	TFQSCIASLLTGAPQPNLGSWKRGSGSMDDSFYDWFPRQLS	6.6	4.7	0.7	6.8	
RP6-0-RP9-IGFR-G8	NCYSSLSGLLDGPPHPPEPFLGRQPFWSGRIFLRLVRLAGCCR	7.2	4.6	0.9	5.2	
RP6-0-RP9-IGFR-B12	TIYSGLASLLRGTPPEANIGLWERRRGTLDSEFYDWFPRQLG	10	4.5	0.4	10.1	
RP6-0-RP9-IGFR-B9	TFYSSLASLFLGTPPLNYGPPVRRPGSLDESFYDWFDSQLG	7.6	4.4	0.9	4.9	
RP6-0-RP9-IGFR-A4	TFQSCLTSLTAAPQPTRAVWEGSRVSRDESFYDWFPRQLS	11.8	4.1	0.7	6.2	
RP6-0-RP9-IGFR-H9	TLQSSLASLVSGSPEPKRVQRERSRGSLLDDSFYDWFDRQLG	9.7	3.9	1	3.8	
RP6-0-RP9-IGFR-C8	TSSLSRASQLTGTLPQNRGQERYRGSLLDDSFYEWFERQLG	4.7	3.6	0.8	4.3	
RP6-0-RP9-IGFR-F3	SFYSCVCSLLNGTPQPTRGLWDRCSGFFDESSDFCFAGELG	3	3.4	0.8	4.2	
RP6-0-RP9-IGFR-A1	DVYSWCGLWLGTTPQAQRVSLLEYRGSRDESFYDWFPRQLG	10.1	3.2	0.5	6.1	
RP6-0-RP9-IGFR-A12	TFYSRQASSWTGALKRNGCPQEGCRGSLDGSFYDWFPRQLG	7.3	3.1	0.4	7.5	
RP6-0-RP9-IGFR-E4	SSTSWPGFALWTGIAQPNLGPWHRLRGSPPDFYDWFQFQLG	12.5	3	0.8	3.7	
RP6-0-RP9-IGFR-D9	TFHSQALTAASCSAPGCGPSLEYRGSLSDESFTWFERQLG	8.4	2.9	0.9	3.2	
RP6-0-RP9-IGFR-F2	TFNSSLASLLTGTPTQATGAPWETWRGQDELFDYDWFPRQLG	8.1	2.8	0.9	3.2	
RP6-0-RP9-IGFR-D8	GFYSCLASLVGTTPQPNRGQVRCRGTLDQVQKFGKGLG	4	2.7	0.8	3.6	
RP6-0-RP9-IGFR-F5	TSQSGLASLLTGSPKSFSGPGERWGGSLDEAFYDWFPRQLG	3.9	2.7	0.8	3.3	
RP6-0-RP9-IGFR-H6	TSSLAWASLLTGSPQLNRGPWESSRGSLLDEQFYEWFEHQLG	4.5	2.7	1.2	2.3	
RP6-0-RP9-IGFR-C12	TGHYWRDFELDGTPEPNRGPLERGGTLDGSFYDWFPRQLA	4.9	2.5	0.4	5.7	
RP6-0-RP9-IGFR-B8	TLDSSLVARLNGTPQNGGPWERIRTSLSDESFYEWFERQLG	3.4	2.5	0.8	2.9	
RP6-0-RP9-IGFR-F4	TLYSCRASLVGTGNARPNNDGPWELCGGSLDESFYDWFPRQLG	7.7	2.4	0.9	2.7	
RP6-0-RP9-IGFR-G7	TFYSQAASFADLYSGQNRLREHYRGSQDESFYDWFPRQLG	4.6	2.2	0.9	2.3	
RP6-0-RP9-IGFR-D12	TFNTWLTPLLNGTQPNAGRWELCSGSQDECFYGWFERQLG	3.1	2.1	0.4	5	
RP6-0-RP9-IGFR-E8	TLQSGLASLVGTGNPKASGGPWGSPRGSLLDGNFYDWFPRQLS	6.8	2.1	0.8	2.6	
RP6-0-RP9-IGFR-D6	TFDSCWASLIPCTPLPNRGWPRGRGSLDKSFYDWFPRQLG	4.7	2	0.8	2.5	
RP6-0-RP9-IGFR-C10	GFYSSLDQMLNGIPQPNRGAWERCRCDCLSFYDWFPRQLG	7.4	1.9	0.8	2.3	
RP6-0-RP9-IGFR-E11	SFYSCWASLSTGTLGGAWERCPSDTSSESFEREPG	5.1	1.9	0.9	2.2	
RP6-0-RP9-IGFR-E6	TLQPCLASLMAGSPTQDRGPWERVPVAPDESFYGWFERQLS	5	1.8	0.6	2.9	
RP6-0-RP9-IGFR-G10	YFYSGLARLPSGTQAQNRGPWEGRGSGSGSFYDWFPRQMG	5.3	1.7	0.9	1.8	

FIG. 61B

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RP6-6-RP9 vs IGFR

Clone #	SEQUENCE	E-Tag	IGFR	LDH	Sp/Irr	SEQ ID NO:
Design	TFYSCLASLLTGTPQNRGPWERCRRGGSGGSLDESFYDWFERQLG	--	--	--	--	--
RP6-6-RP9-IGFR-C5	VMGSESMDDSFYDWFERQLG	48.9	22.5	1	23	23
RP6-6-RP9-IGFR-D12	TLSSSRASLQAGAPQNRGRWERCGLVSGSGSLDDSFYEWFERQLG	46.2	26.4	1	27	27
RP6-6-RP9-IGFR-G4	TFYSGVASLMTGTTQNRGPWERSRGEARGSDSLDASFYDWFERQLV	40.8	21.1	0.8	28	28
RP6-6-RP9-IGFR-H8	RSGPGSVDDTFYEWFERLLG	41.8	16.1	0.8	19	19
RP6-6-RP9-IGFR-D10	NFYSYQPSLADWYPGSPTGLHGDVAVALMVAGAGSLGDSFYEWFERQLG	46.5	25	1	26	26
RP6-6-RP9-IGFR-C11	ISSCLAFSADWLLQRPVSWQEGRRGGYGGGSLHDSFYGWFERQLG	15.7	3.3	0.8	4	4
RP6-6-RP9-IGFR-A12	GLGSSGRDESFYDWFERQLG	48.3	30.6	0.8	37	37
RP6-6-RP9-IGFR-H10	VLGGSGALDESFYEWFERQVG	48.1	22.7	1	22.1	22.1
RP6-6-RP9-IGFR-G9	WSVGYVGRSGLGSDSQDESFYDWFERQLG	41.6	22.2	1	22.2	22.2
RP6-6-RP9-IGFR-H3	VVVGSGSLDESFYDWFERQLG	51.3	33.6	1	35.2	35.2
RP6-6-RP9-IGFR-A10	VSGSGTRDESFYDWFERQLR	35.3	24.9	0.6	38.7	38.7
RP6-6-RP9-IGFR-E12	VRGISGFPEDSFYGWFERQVD	47.7	35	0.8	41.6	41.6
RP6-6-RP9-IGFR-C6	VPGGSGSLDESFYAWFERQVG	49.2	27.5	1.1	25.5	25.5
RP6-6-RP9-IGFR-E11	VLVAQSLDESFYAWFERQLG	33.5	14.2	1	14.7	14.7
RP6-6-RP9-IGFR-F1	VLRGASQDESFYHWFERQVA	47.8	30.4	0.9	32.7	32.7
RP6-6-RP9-IGFR-A2	VLRAPGSLDESFYEWFERQLG	51.3	32.6	1.1	30.7	30.7
RP6-6-RP9-IGFR-A11	VLGSVSLDESLYAWFERQLG	44.8	33.3	0.8	39.9	39.9
RP6-6-RP9-IGFR-F10	VLGRSELLNESFYDWFERQLG	44.8	26.2	1	25.8	25.8
RP6-6-RP9-IGFR-B5	VLGGALDASFYDWFARQLG	48.1	25.3	1	25.8	25.8
RP6-6-RP9-IGFR-B8	VLARSSSLDEAFYDWFERQLG	47.9	29.2	1	29.8	29.8
RP6-6-RP9-IGFR-A1	VGWVVGSLDEQFY*WFERQLG	16.6	10.6	1	10.8	10.8
RP6-6-RP9-IGFR-B3	VACGSDSMDECFYDWFERQLG	33.5	9	1.1	8.2	8.2
RP6-6-RP9-IGFR-F7	TVYPTPASLLDGSPTNRARWKRLDGGSGSGALDESFYHWFEDQLR	39.8	12.7	1	12.9	12.9
RP6-6-RP9-IGFR-C9	TSLRLRETVAVGTRGSGALEESFYDWFERQLG	45.8	16.6	0.9	18.2	18.2
RP6-6-RP9-IGFR-B10	TLYPWPGFYADWYSGAGPVARGNVGGSGSGSLNESFYEWFERQLG	27.1	6.9	0.9	7.8	7.8
RP6-6-RP9-IGFR-C8	TFYTSLAPQLTGPPQPHRGPSARGRVGGSGSGSLDESFYGWFERQLG	21	4.9	1	5.1	5.1
RP6-6-RP9-IGFR-B4	TFYTCLAALVTGNPQNRGPWERCARGSGSGSLDEFYDGFERQVG	4.6	1.5	1.1	1.4	1.4
RP6-6-RP9-IGFR-E3	TFYTCLAALVTGNPQNRGPWERCARGSGSGSLDEFYDGFERQVG	2.6	1.4	1.1	1.3	1.3
RP6-6-RP9-IGFR-E2	TFYSCLTSLQTVTPSPNPGSWERSRGGSGSGSVQESFYDWFERQLG	55.1	16.9	1	17.3	17.3
RP6-6-RP9-IGFR-A3	TFYSCLDALLAGPEPWCRCRSPGGLGSLDGLSLDWLEGERG	7	1.8	0.9	2.1	2.1
RP6-6-RP9-IGFR-A5	TFYSCLAALLTAPPQNGGAWERCRCFGYLDDESASDVQSOLA	11	3.5	0.9	3.7	3.7
RP6-6-RP9-IGFR-E8	TFYS#LPSLLTGTKPSGGPWERGRNVGGTGSLSNEAFYDWFERQLG	25.2	6.3	1	6.4	6.4
RP6-6-RP9-IGFR-D7	TFYPCQGSFAGLVPLARTVGRGNVARAGSVGGSLDGSFYDWFERQLG	45.4	13.4	1	13.4	13.4
RP6-6-RP9-IGFR-D8	TFTSGLRYL.PGLSQASVSDRSRGGSGSGSLDESFYKWFERQLG	16.4	2.9	0.9	3.2	3.2

FIG. 62A

Clone #	SEQUENCE	E-Tag	IGFR	LDH	Sp/Irr	SEQ ID NO:
RP6-6-RP9-IGFR-G5	TFQYCLGAVLSDTPQVNGRSPDRGGGGSGPGSLDEFFYGFWERQLG	7	2.4	0.8	2.9	
RP6-6-RP9-IGFR-B2	TFQTCMANRSGTPERNGSLGWRASSGGSGSLDESFYDWFDRQLG	35.6	10.6	1	10.3	
RP6-6-RP9-IGFR-B9	TFQSSVASLLTGTLPHRGPWDRLRGGSGVSGSLDESFYDWFDRQLS	4	1.4	1	1.5	
RP6-6-RP9-IGFR-D5	TFNRIQSTLLTGTMQPIRAPKECCRWARGSGSVGESFYDWFDRQLG	1.2	1.2	1	1.2	
RP6-6-RP9-IGFR-G11	SSSFPFGSGLVLRSGTVVRGDVTLVPAAVNLDESFYDWFDRQLG	43.9	27.8	1	28.8	
RP6-6-RP9-IGFR-G7	SFYSWLGSADQGSATEQWPRWDVAGVASRGTGSLDELFIYDWFDRQLD	22.5	9.1	1.1	8.2	
RP6-6-RP9-IGFR-H5	SFYSGLSLLTGRPPQNRCAWERCRCGGSGGSLDEGFYDWFDRQLG	30.9	21	0.9	24.3	
RP6-6-RP9-IGFR-H7	SFYSALGSLLPGTQPGRAGGGSGGSLDESFGWFERQLG	41.3	29.1	0.8	37.9	
RP6-6-RP9-IGFR-D11	RSYGGPLDESFEWFERQLG	46.1	25.4	0.8	32.4	
RP6-6-RP9-IGFR-F4	PYTSDRGLADQSSAGRNVRGRGWDASGSPHDSFYDWFETQLG	11.5	2.8	1	2.7	
RP6-6-RP9-IGFR-C12	NVH#SLDGLLSCASQPSRGPWGRWLGDGSGGSLDSSFYDWFDRQLG	29.5	6.9	0.9	7.6	
RP6-6-RP9-IGFR-C7	NFGAGSLDESFYDWFARQLA	14.4	4.1	0.9	4.5	
RP6-6-RP9-IGFR-E5	NFESCGASVMAGTPQNYRRPWERTRGSSSGTLDSEFYDWFDRQRG	43.2	6.7	1	6.7	
RP6-6-RP9-IGFR-E10	MTGPAQRNVLWEGWPGVSGSGSMDDSFYGFWRQLG	40.7	16.6	1	17.2	
RP6-6-RP9-IGFR-F9	MSTLGLAFLRTGTTPQNRGPWGHGGSGGSLDSDSFYDWFDRQLG	43.6	20.1	1.1	17.8	
RP6-6-RP9-IGFR-D9	ILSPASLLVPTDTPQAESWIRGDRGSSSGAGSQERSFYDWFDRQLG	32.7	4.9	0.9	5.3	
RP6-6-RP9-IGFR-B1	HLVALSSLDQSFYAWFERQLG	16.5	7.9	0.9	8.4	
RP6-6-RP9-IGFR-F8	HFYSALGSRGLERDRGESGGSGRDESFYDWFDRQLG	45.1	24.9	1.1	23.6	
RP6-6-RP9-IGFR-D3	GYRATGSLDGSFYEWFERQLG	50.3	33.9	1	35.3	
RP6-6-RP9-IGFR-G8	GSYSGLATRQIGNQQPNGQWETGRGSRGSGSKDESFYDWFDRQLG	31.4	7.5	1.1	6.9	
RP6-6-RP9-IGFR-E9	GSVRSGLDSDSFYEWFERQLG	41	26.3	0.8	31.4	
RP6-6-RP9-IGFR-D1	GSVGSASLDKAFYDWFDRQLG	32.6	14.3	1	13.8	
RP6-6-RP9-IGFR-B6	GSRGSGSLDTSFYDWFDRQVG	52.6	28.1	1	27.6	
RP6-6-RP9-IGFR-C2	SGSVSGSLDESFYDWFDRQLR	49.2	33	0.9	36.6	
RP6-6-RP9-IGFR-F3	SGSRSGFQDSAFYEWFERQLG	50.1	29.5	1	29.5	
RP6-6-RP9-IGFR-C10	SGSRGSLDESFYDWFDRQVS	45.1	30.5	0.9	34.1	
RP6-6-RP9-IGFR-E6	SGGHQDFLEESFYDWFARQLA	26.7	8.1	0.7	11.8	
RP6-6-RP9-IGFR-F11	SGGGYASRDESFEWFERQLA	52.4	36.5	0.9	40.7	
RP6-6-RP9-IGFR-F6	SGGGSGTLDEYFYDWFDRQLR	0.9	12.2	1	12.4	
RP6-6-RP9-IGFR-F5	SGGGSGSLNASFYEWFERQLS	37.2	13.2	1.1	12.3	
RP6-6-RP9-IGFR-B7	SGGGRGSLDESFYDWFDRSQLG	50.1	31.9	1	33.2	
RP6-6-RP9-IGFR-G3	GRGGTSLDASFYEWFERQLG	55	36.1	1	37.9	
RP6-6-RP9-IGFR-D2	GLGGSGSKDESFEWFERQLS	50.8	36.8	1	38.4	
RP6-6-RP9-IGFR-G1	GGVSGSRDESFYDWFARQLA ₂	47.6	35.5	0.9	40.1	
RP6-6-RP9-IGFR-C1	GASGAGSLDKSFYAWFARQLD	29.7	11.6	1	11.6	
RP6-6-RP9-IGFR-E7	ESFYEWFERQLG	32.2	19	0.6	29.9	

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FIG. 62B

Clone #	SEQUENCE	E-Tag	IGFR	LDH	Sp/Irr	SEQ ID NO:
RP6-6-RP9-IGFR-A6	DSFYEWFERQLG	38.7	18.4	1	18.4	
RP6-6-RP9-IGFR-H4	DLVGLGSLNESFYDWFERQLG	46.4	30.1	0.9	32.9	
RP6-6-RP9-IGFR-B11	DIYLAGYPADRYATEPLGRWERSRRSGSGSSSLDESFYNWFERQLG	42.9	17.9	0.8	22.5	
RP6-6-RP9-IGFR-B12	DHLLPCAASSAALGTP*PSRGSVGALPGAVLDGFGSIDESFYEWFEQLR	26.5	6.9	0.8	8.3	
RP6-6-RP9-IGFR-D6	DFSTLAWESLLDWSSVQWPVRGERGRGGSGVTGSLDQSFYDWFERQLG	28.6	6.3	0.7	8.5	

FIG. 62C

D815-RP6 vs IGFR

CLONE	Design	Sequence	Etag	IGFR	LDH	IGFR/LDH SEQ ID NO:
D815-RP6-IGFR-4-D10 ₂		WLDQERAWLCEISGRGCLSTFYSCLASLLTGTGPQNRGPWERC	--	--	--	--
D815-RP6-IGFR-4-G10 ₂		RSTFYSCLDLTLLTGAPRGNRGWECWG	12.3	14.1	1.0	13.6
D815-RP6-IGFR-4-E11		GQFSGGTVSSFYSCIGSLTSGPKSRGPWESCR	13.9	14.0	1.2	11.4
D815-RP6-IGFR-4-C10		WPAREISAGGRQSTFYACLSLLSGSLQGTGAPWERC	12.5	13.8	1.0	13.8
D815-RP6-IGFR-4-C5		GGFYSCLDLTLTGSPQKRGWERC	13.3	13.2	1.0	13.2
D815-RP6-IGFR-4-G11		WADRERDSLWRANSSRGYRSTFYSCLSLDSLLSGTPQKGGPWERC	11.4	12.5	1.0	12.5
D815-RP6-IGFR-4-D3 ₂		SSFYSCLTSLVTGSRQWNGGPDRCRA	11.9	12.1	0.9	13.3
D815-RP6-IGFR-4-B4		QMGSFYSCLSNLLSGTPQDRGPWAGCR	9.9	12.0	0.9	13.5
D815-RP6-IGFR-4-B9		SSFYSCLASLLTDNPRAGRGWERC	11.0	11.9	1.0	11.9
D815-RP6-IGFR-4-E4		FGSGGSTFYSCLASLLTGAPQNRGAWERC	11.2	11.9	0.9	13.0
D815-RP6-IGFR-4-C8		AWDQERGWPRSTFYSCMASLLTGNPQNRGPWEGCR	12.4	11.7	1.0	11.9
D815-RP6-IGFR-4-C12		GMVKGAWLRDESSGRGSRSPFYSLWDCLVTVTPQADGGAWERC	11.0	11.6	0.9	13.5
D815-RP6-IGFR-4-C4		NSGRGSVSTFYSCLDLSSGTQANGGLWERC	12.3	11.5	1.0	11.3
D815-RP6-IGFR-4-B10		SSFYSCLASLLTDNPRAGRGWERC	10.8	11.4	1.0	11.9
D815-RP6-IGFR-4-D5 ₃		QLDKEGAWPRCDNSGRGCRSTFYSCLSLVTGTGTEQLKRGPWELCR	9.4	11.2	1.0	11.0
D815-RP6-IGFR-4-D7		FRGGQSTFHSCLFSLSSSTPRDNRGPWGRCR	10.2	11.0	0.9	12.7
D815-RP6-IGFR-4-C6		WQOQGGKALGAGDVNFYSCLSLTLTGTPEANRGTWEGCR	9.8	10.7	0.9	11.6
D815-RP6-IGFR-4-B3		WAGFYGCLASLVTGTPRPQGGPGEGRH	9.7	10.5	0.8	13.3
D815-RP6-IGFR-4-E12		QGWVTSFYSCIGSLLAGTSQAKGPGWQRCR	11.5	10.5	0.9	11.6
D815-RP6-IGFR-4-E8		LVDNRFYCGLASLLTGTTPRQNGSPQGRCL	11.0	10.3	1.1	9.7
D815-RP6-IGFR-4-E3		QIGSETFYSCLTSLLRGTGPQNRGPLERCR	10.0	10.3	0.9	11.5
D815-RP6-IGFR-4-C7		DTFYSCLASLVTGTRAENRGPVSCR	10.6	10.2	0.9	11.4
D815-RP6-IGFR-4-E2		WMEQESAGLRTEISGRRSQSFYSCMDLSLSSGNAESDGRQWERC	11.4	9.9	0.9	11.0
D815-RP6-IGFR-4-F12		SSFYSCLTSLVTGSRQWNGGPDRCR	8.6	9.9	0.9	10.5
D815-RP6-IGFR-4-E10		WAELEARLWREMSGGGLTGFYSCLASLVTGTPLANRGSWERC	11.2	9.8	1.2	8.5
D815-RP6-IGFR-4-D11		STFYSCLASLLPGHREPSGGPDRCR	11.1	9.8	0.9	10.5
D815-RP6-IGFR-4-A6		CTFYSCLASLLTGTPEQNRGWEECCR	12.4	9.8	1.0	9.8
D815-RP6-IGFR-4-B6		VAHRSTFYSCLGALLTGFPRENDRDSWESCR	8.4	9.5	1.1	9.1
D815-RP6-IGFR-4-B12		WSGLSGGSTFYSCIGSLTGTTPRNLDWEGCR	8.8	9.4	0.8	12.2
D815-RP6-IGFR-4-A4		STCVGCEISGRSRLRTFYSCLDLALVTGSAQPNRRPWERC	10.3	9.4	0.9	10.2
D815-RP6-IGFR-4-C2		WLGQERAPATFYSCIGLALLTGPPQNRGPWDGCR	5.9	9.2	1.1	8.4
D815-RP6-IGFR-4-G9		RLDQDPVSGGERSGRSLSFVACLGLSLVTGAQNRGPWERC	10.3	9.2	1.0	9.6
		QLDRDFYCLDALLTGTAQNGGPWRCR	10.7	9.2	1.0	9.4

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FIG. 63A

CLONE	Sequence	Etag	IGFR	LDH	IGFR/LDH	SEQ ID NO:
D815-RP6-IGFR-4-E9	WPDLERAGPRGEI . GRGGRSTFYSCLASLVAGTAQPNRGPWRCR	10.8	8.7	0.9	9.3	
D815-RP6-IGFR-4-E5 ₂	WMDLSVVASRSTFYSCLSLLTGAPLQNRGLWRCR	8.3	8.7	0.9	9.3	
D815-RP6-IGFR-4-D6	VGGTFYSCLASLLTGSPPNRGAWRCR	9.0	8.5	0.7	11.5	
D815-RP6-IGFR-4-G4	SSFYSCLTSLVTGSRQWNGGPDRCR	7.8	8.5	0.8	11.0	
D815-RP6-IGFR-4-G3	SNFYSCLDLSLLTGFPQNGSQWEGCR	8.2	8.5	0.8	10.8	
D815-RP6-IGFR-4-B7	GLDQDRAWLWSGLSPRAGSTFYSCLASLLTGTPQPNRGPARGCR	8.9	8.5	0.9	9.4	
D815-RP6-IGFR-4-A10	NSAVVGSLSSTFYACIASLVKGTQQSPWRCR	9.3	8.4	0.9	9.8	
D815-RP6-IGFR-4-G7	TLDPSTFYSCLASLMTGTLPPNHLSERCR	9.1	8.0	0.9	9.3	
D815-RP6-IGFR-4-C11	SQDQVGAISGSAISGRGGLSNFYSCLSLTLATDTTPSRRPWRCR	12.2	8.0	1.0	8.3	
D815-RP6-IGFR-4-B1	RLQGERAWFWGESSGRGGLGSFYGCLADLVRGNPQANSQPGRCR	6.6	8.0	1.0	8.0	
D815-RP6-IGFR-4-E1	WRPSFNSCLDSLLTGRGRPSGGLWRCR	8.0	7.9	1.0	7.8	
D815-RP6-IGFR-4-A11	TFYSCLESLLTGSPQPNRGAWRCR	8.3	7.8	0.6	13.4	
D815-RP6-IGFR-4-F2	GLQGEAQLSCMSDFTFYSGLSLLTGTRQNSGTWERFH	10.1	7.8	1.1	7.2	
D815-RP6-IGFR-4-G2	QVDRERAWPGCDLSGRGSLDAFYSGLASLLTATRQNRGPWELCG	7.2	7.7	1.0	7.6	
D815-RP6-IGFR-4-C1	SGFYSCLASLMAGTTQNRNYQLEGCR	7.2	7.6	1.0	7.5	
D815-RP6-IGFR-4-A2	LPVSFYSCLASLQTGAPQPSRGPWRCR	7.2	7.4	1.0	7.1	
D815-RP6-IGFR-4-A5	GGSTFYSCLTSLLAGTPRPRGDLWGSCV	9.3	7.4	1.1	7.0	
D815-RP6-IGFR-4-B2	WGGSRSTFYSCLDLSLLRTAPQNGGQWDGCF	10.0	6.7	0.9	7.3	
D815-RP6-IGFR-4-G6	WTEETRGWVRN . GRLATFYSCLDLSLLTVTSQPDPRGPWRCR	10.3	6.5	0.9	7.0	
D815-RP6-IGFR-4-A8	SAFYSCLASLLTGTPRPNNGGPDWDCR	9.8	6.4	1.0	6.6	
D815-RP6-IGFR-4-A1	WAGPGTFSFYSCLASLLTSTSQPKRGLWRCR	5.2	5.3	1.0	5.2	
D815-RP6-IGFR-4-H2	WMDQGRLLTTFESCINSLLTGICQPNRGPWRCR	6.3	4.2	0.8	5.0	
D815-RP6-IGFR-4-G1	GQDRERAQLWCEISSGGTFYSGLASLLSGTREPTRGPTRCR	5.6	4.1	0.9	4.7	
D815-RP6-IGFR-4-H11	VTFYSCLASLGAAGTPQPNRGPWDCR	8.2	3.4	1.0	3.4	
D815-RP6-IGFR-4-D9	QLDQGRAWLWCEISGSGCLPTFESCLATLLTGTPERNRPWDSCR	4.8	2.0	1.0	2.0	

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FIG. 63B

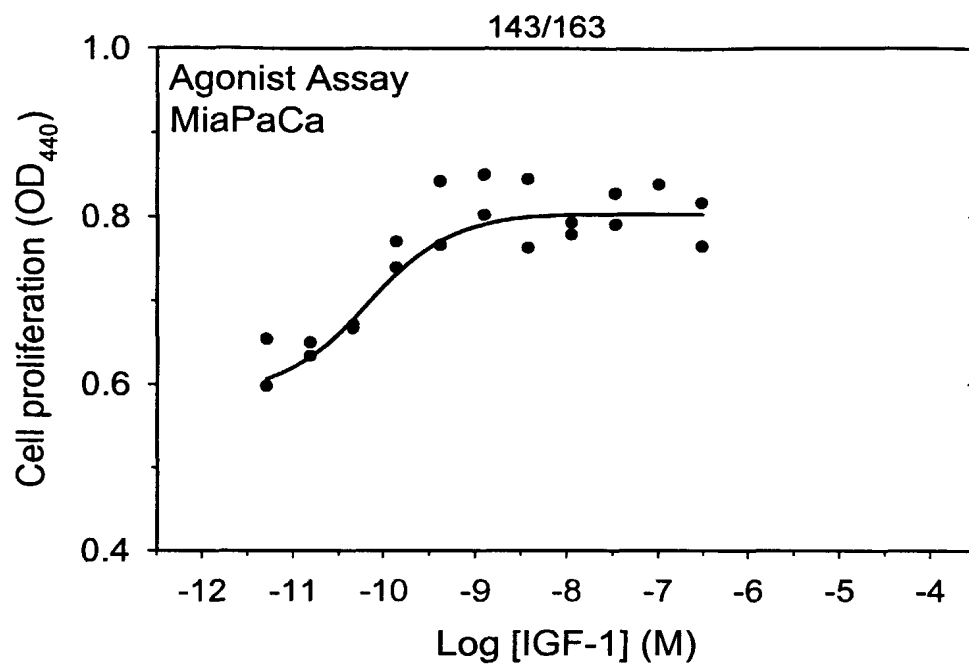
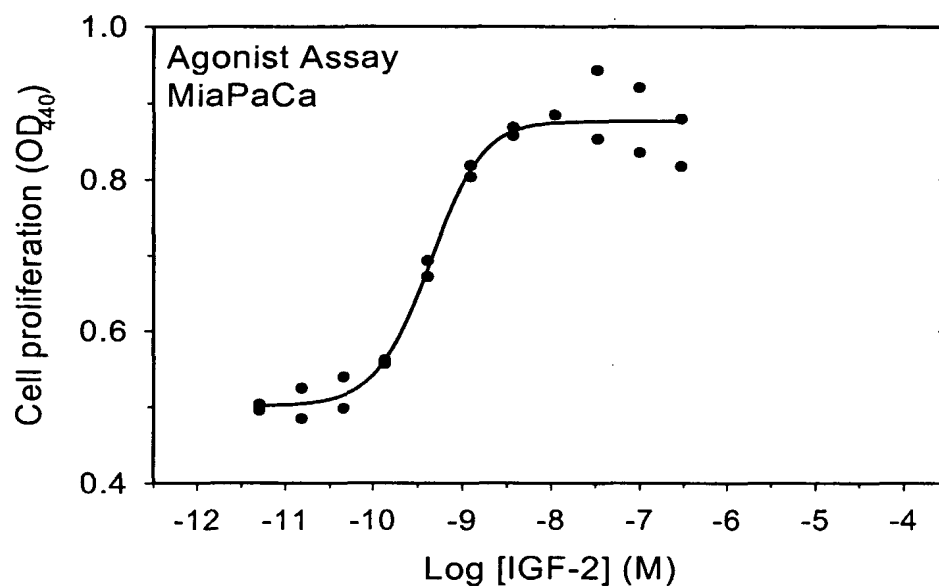
D815-6-RP6 VS IGFR

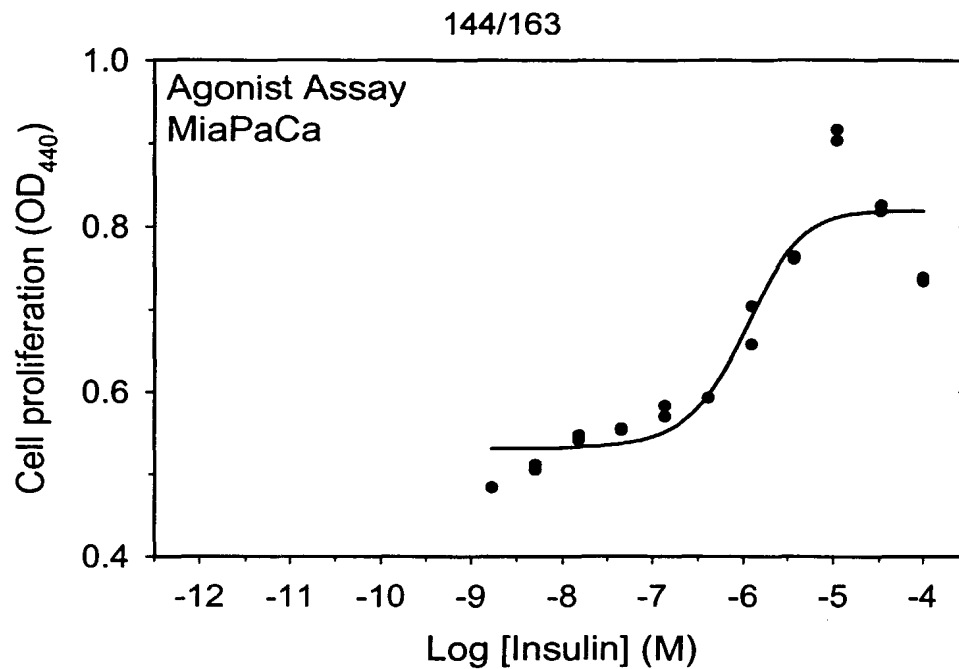
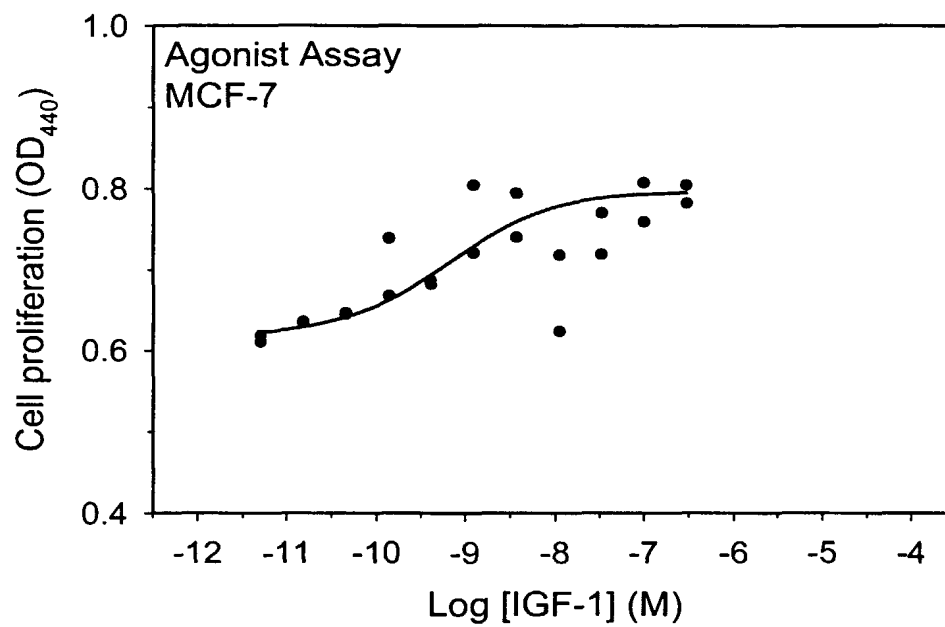
Clone	Sequence	Stag	IGFR	LDH	IGFR/LDH	SEQ ID NO:
Design		--	--	--	--	
D815-6-RP6-IGFR-4-H10	<u>WLDQERAWLWCEISGRCLSGGSGSTFYSCLASLLTGTPQPNRGPWERC</u>	14.9	17.7	1.3	14.1	
D815-6-RP6-IGFR-4-D11	<u>SVEPENKGGGLKSFYSCLASLLSGTPEODRGPWEGCR</u>	12.7	17.4	1.4	12.8	
D815-6-RP6-IGFR-4-D9	<u>SSFYSCLASLLTDTQPRRGKWERC</u>	13.4	17.3	1.6	10.7	
D815-6-RP6-IGFR-4-D1	<u>QNLSPWLGGSGGSHFYSCLASLLTGTPQPNRGTGDRCR</u>	12.5	17.0	1.4	12.4	
D815-6-RP6-IGFR-4-E8 ₂	<u>SLDQQRAGRTFYSCLATLLMGTPQPNGGPWKDCR</u>	12.5	16.1	1.4	11.3	
D815-6-RP6-IGFR-4-B9 ₂	<u>GLQERGGSTFYACLASLVTGSGQANHGLGERCR</u>	11.5	15.8	6.9	2.3	
D815-6-RP6-IGFR-4-D5	<u>SLLSGGSTFYSCLASLRTGGPQANRGPWERC</u>	12.4	15.8	1.2	13.1	
D815-6-RP6-IGFR-4-C6	<u>RLDRERPWVSLDSSVRGWLGGSGGSTFYSCLSLLADTPERNRGSWKRCR</u>	13.2	15.7	1.5	10.4	
D815-6-RP6-IGFR-4-B4	<u>WDRTRSGGSSFYSCLSLLGGFTQDRGQWEGCR</u>	11.6	15.6	1.3	12.3	
D815-6-RP6-IGFR-4-E12 ₃	<u>WQDPDRAGLWRVSYGRG.VFGGSGGSSFYSCLASLVSGTRQPNRGPWERC</u>	10.6	15.6	1.0	16.0	
D815-6-RP6-IGFR-4-B5	<u>TGGGGSTFYSCLVALLGGPPQKGAWGRCR</u>	12.9	15.6	1.2	12.8	
D815-6-RP6-IGFR-4-H12	<u>QARPGSGGSSFYSCLAALLTGDAQPNRGSVRRCR</u>	12.1	15.5	1.1	14.2	
D815-6-RP6-IGFR-4-D7	<u>RGSTFYSCLSLLTGTPHRDRGPWERC</u>	11.7	15.4	1.2	13.2	
D815-6-RP6-IGFR-4-B6	<u>LVEQERAGLGCIEIACRCLSGGSGSTFYSCLASLINGTLQSNRGATEGCR</u>	12.1	15.4	1.4	11.2	
D815-6-RP6-IGFR-4-E10	<u>QVDQDSSGSTFYSCLASLLTGATQPSGGPWERC</u>	14.2	15.2	1.6	9.7	
D815-6-RP6-IGFR-4-D8	<u>WAAKEGASFYSCLASLLNGTLPTRGPWERC</u>	12.2	15.1	1.4	11.1	
D815-6-RP6-IGFR-4-C3	<u>RVGEISSRGFLAGSGGSGSTFYHCLASLLTWTPEANGDPWERC</u>	12.0	15.0	1.3	11.2	
D815-6-RP6-IGFR-4-E6	<u>WRDKEQAGLWSEISGRGSPSGGSGSTFYACLAALLSGTQEPNSGKWERC</u>	11.8	14.9	1.4	10.5	
D815-6-RP6-IGFR-4-F7	<u>WQDEERPWTWREGSGRGLSGGSGSTFYSCIAALLHGTPOANRGTWEGCR</u>	11.1	14.7	1.5	9.5	
D815-6-RP6-IGFR-4-A5	<u>WMDQGRATWIEISGRGLSGGSGGSSFYSCLASLMTGAPLPKRGAWEP</u>	11.5	14.6	1.2	12.7	
D815-6-RP6-IGFR-4-B8	<u>SVDQERGAISGRGLSGGSGGSTFYSCLASLLTGTPKDRGPWEGCR</u>	12.2	14.3	1.4	10.2	
D815-6-RP6-IGFR-4-F12	<u>WLEQSEIARRGWLSGGSGGSGSTFYSCLSLLTGTEQNQGSPVACR</u>	10.1	14.2	0.9	15.7	
D815-6-RP6-IGFR-4-E3	<u>WRDEERGAWAGREISGRGLSGGSGGSGSTFYSCLSLLIGTRQPGRGAWEP</u>	10.9	14.2	1.2	11.9	
D815-6-RP6-IGFR-4-C4	<u>GVEQERDWLGGISGRGLSGGSGGSGSTFYSCIASLLGGTTPHPERGSWERC</u>	11.3	14.1	1.3	11.0	
D815-6-RP6-IGFR-4-D12	<u>WVNQDRSGGSGSFYSCLASLLNGTAKPYRCR</u>	10.6	14.1	1.1	12.5	
D815-6-RP6-IGFR-4-C5	<u>GLDPQGGSTFYSCLAYLLAGTPQWNRGPGERCR</u>	10.5	14.0	1.7	8.0	
D815-6-RP6-IGFR-4-C11	<u>WLDLEMLSGGSGSTFYSCLASLMRGPQPNRGPWGCSCR</u>	11.3	13.7	1.4	10.1	
D815-6-RP6-IGFR-4-F6	<u>RVDQERGGGSAFYSCLAYLLTGPPQGRGPLERCT</u>	10.7	13.5	1.4	10.0	
D815-6-RP6-IGFR-4-C2	<u>WMDQERGLRGLSGGSGGSSFYSCLASLLTGPPQCRGAGEGSR</u>	10.8	13.4	1.3	10.0	
D815-6-RP6-IGFR-4-E9	<u>SGSGGSTFYSCLASLLTGTSQPGRGPRVRCR</u>	11.1	13.0	1.5	8.9	
	<u>WLDQESAGGSGGSGSTFYSCLASLLNGTPOPNRGPWERC</u>					

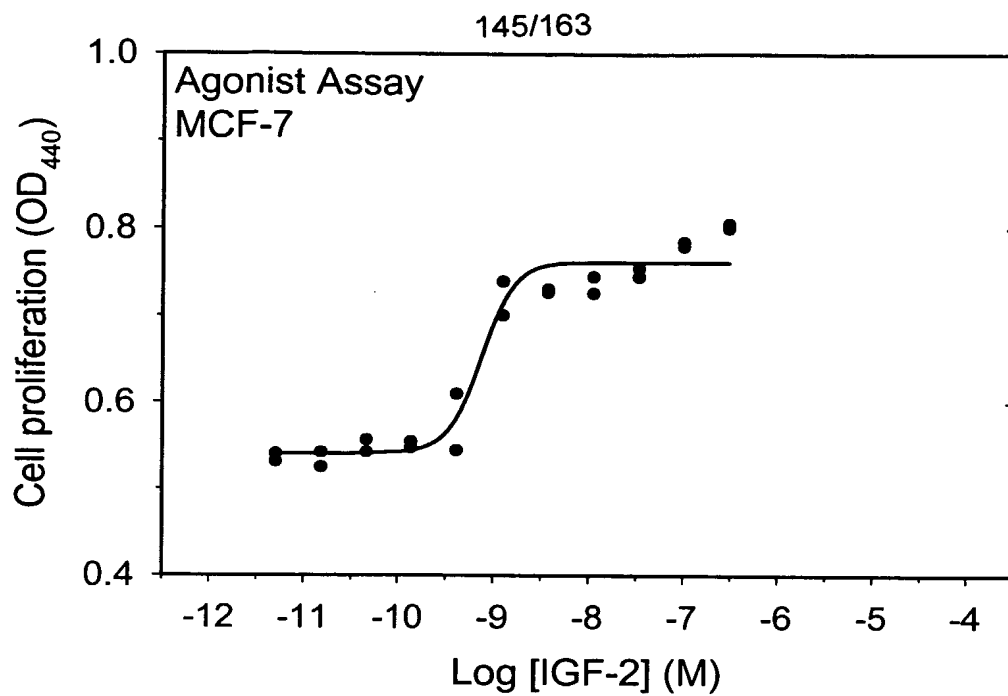
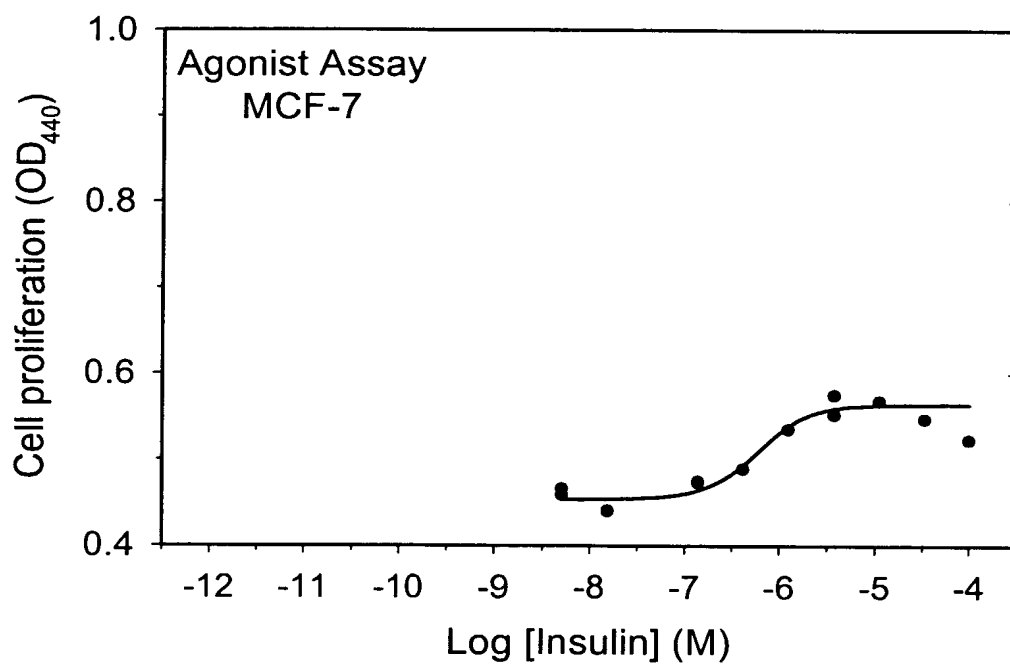
FIG. 64A

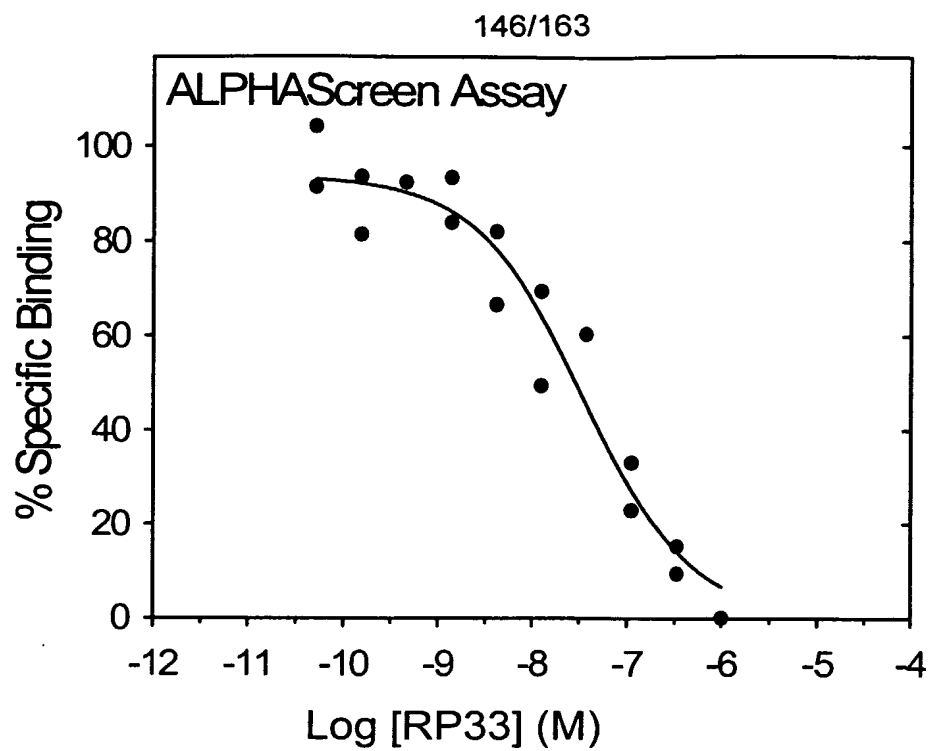
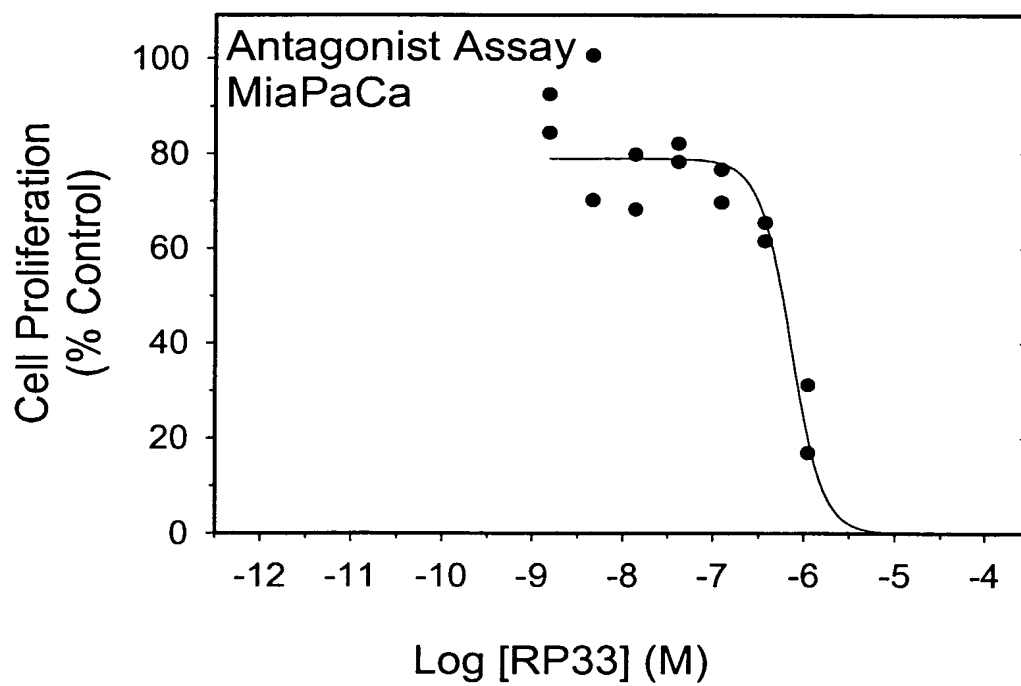
Clone	Sequence	Etag	IGFR	LDH	IGFR/LDH	SEQ ID NO:
D815-6-RP6-IGFR-4-A3	STFYTCLASLLTGAPAPKRGAWERC	7.9	13.0	1.2	10.6	
D815-6-RP6-IGFR-4-D2	GSEQDRAGVQSKI VGRGRHSGGSGGFYSCSLADLLTGTLNRGLWDRCR	9.2	13.0	1.3	10.3	
D815-6-RP6-IGFR-4-E11	WAGPGTCFGRGCVSGGSGSTFYSCSLALPGTQPNRGPQDGCR	11.9	12.9	1.3	10.2	
D815-6-RP6-IGFR-4-A8	SPSSCLASLVSGTWPNRGLWERC	10.5	12.8	1.3	9.9	
D815-6-RP6-IGFR-4-F5	WGSTFYSCSLALLTGTPOAKRGWERC	8.9	12.6	1.0	12.8	
D815-6-RP6-IGFR-4-E5	FRQERAGVRGETSGLGSLSGGSGSTFYSCSLALITGIPQPNRGAWVRCR	8.0	12.6	1.2	10.2	
D815-6-RP6-IGFR-4-D4	QVDQERARLGGENSGRGFAPGGGGSTFYSCSLDLSLLTGTPMSNRGAWERC	10.7	12.5	1.1	11.5	
D815-6-RP6-IGFR-4-B1	GAKAVVGRSGGSGSTFYSCSLALITGTAPDNRGPWEHCR	7.5	11.9	1.3	9.5	
D815-6-RP6-IGFR-4-A4	QLAHERAWMPGGSGSTFYSCSLALLTVTRQLNGRWERC	9.7	11.1	1.1	9.7	
D815-6-RP6-IGFR-4-H8	SLDQERAWLRGEISGRSGGSGGSGSFYSCSLALISGTWQPGGGGDRCR	8.3	10.9	1.4	7.6	
D815-6-RP6-IGFR-4-E2	QNDQSTFYSCSLALMTSTPRPERRPWECRG	8.8	10.9	1.3	8.4	
D815-6-RP6-IGFR-4-F1	WMVQEFAWLGGDISGRGSVSGGSGSTFYTCLAALVSGTP.TNRQWERC	9.0	10.5	1.2	8.5	
D815-6-RP6-IGFR-4-H7	QLEKESGGSGSTFYCWLRSLVTGTPHSDGSTWERC	10.3	10.4	1.8	5.9	
D815-6-RP6-IGFR-4-G1	RLEQEGVGCGSKSVGGCLAGGSGSTFYSCSLAALLTGTPEGNRGAWDRCS	5.7	10.1	1.0	10.5	
D815-6-RP6-IGFR-4-A6	QLDPDGGSTFYSCSLSSLSSTGTNRGPWDRCR	12.8	10.1	1.2	8.7	
D815-6-RP6-IGFR-4-G12 ₂	SGGSTFYSCLVGLVLLTGTPOANRGWEHCR	8.9	9.0	1.0	9.3	
D815-6-RP6-IGFR-4-C1	RAQQERGGLSSEFSRGLLSGGSGSNFYSCSLATLLTGTPLPKRGASEGCR	9.4	8.3	1.4	6.0	
D815-6-RP6-IGFR-4-H2	WGGSGSTFYSCVASLIAGGPQPNRGVWERC	6.6	7.5	0.9	8.8	
D815-6-RP6-IGFR-4-A1	QLGGSSFYSCSLALITGTPOAYRGSWERC	7.8	6.5	1.5	4.3	
D815-6-RP6-IGFR-4-H4	WLGQERRWQGEIVGRGGLSGGSGGFYSCSLSLGRSPERTRGPWELCR	5.6	6.0	0.8	7.2	

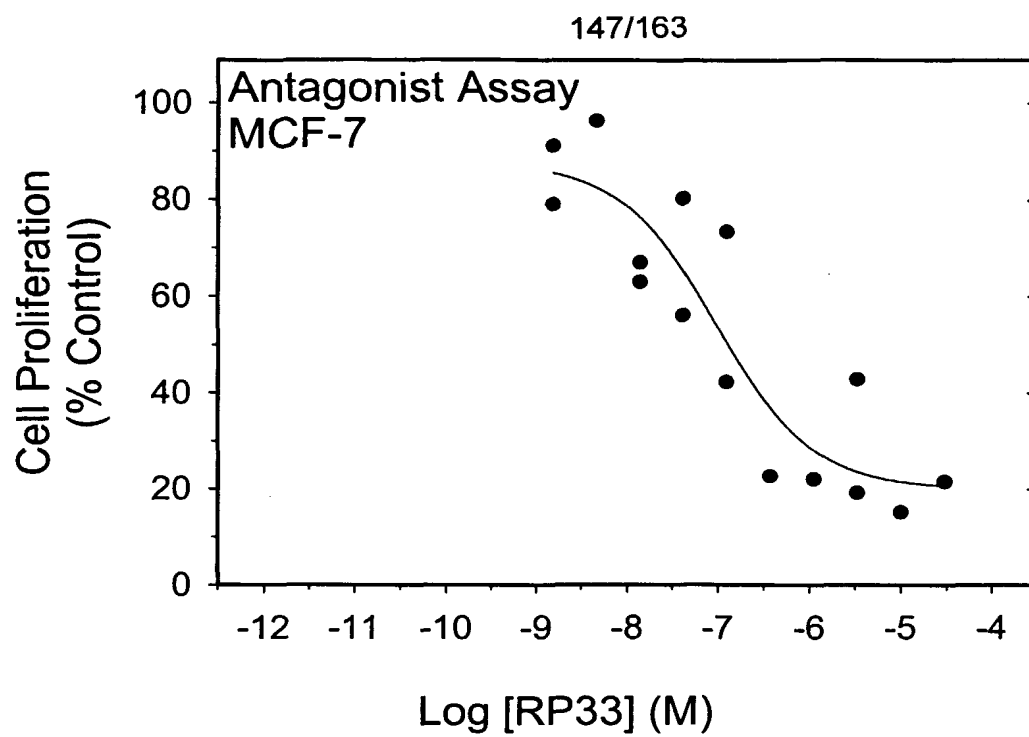
FIG. 64B

**FIG. 65A****FIG. 65B**

**FIG. 65C****FIG. 65D**

**FIG. 65E****FIG. 65F**

**FIG. 66A****FIG. 66B**

**FIG. 66C**

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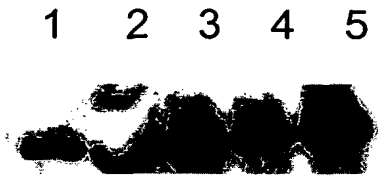


FIG. 67A

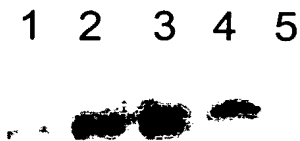


FIG. 67B

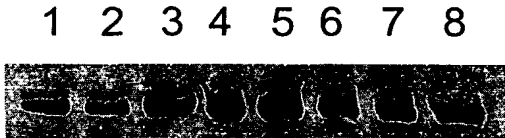


FIG. 68A

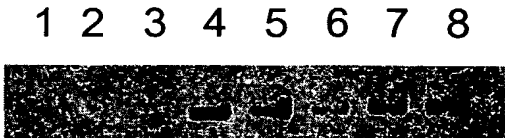


FIG. 68B



FIG. 69A

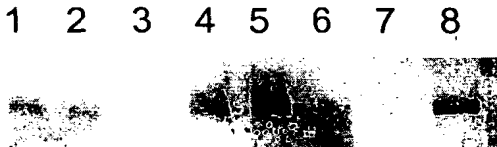
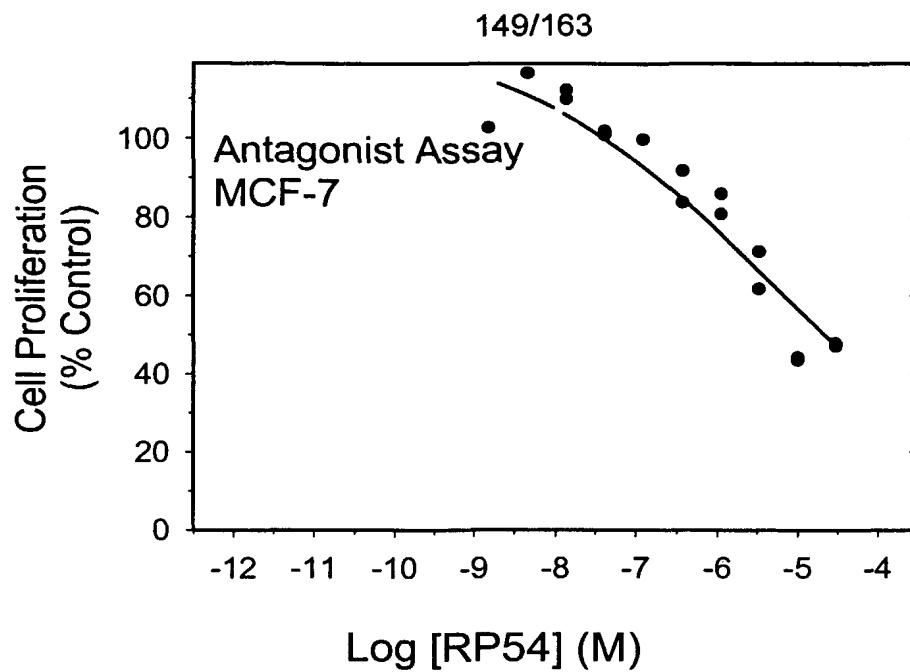
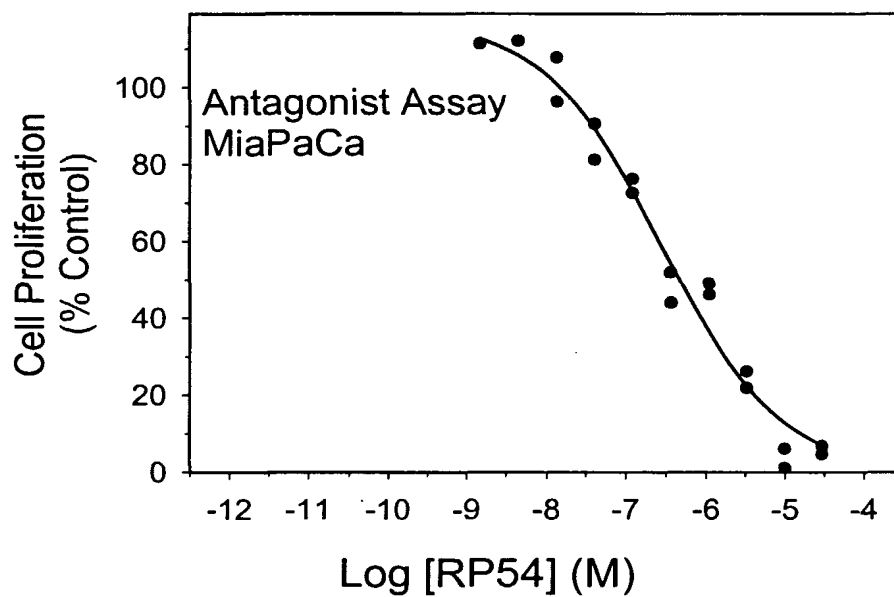
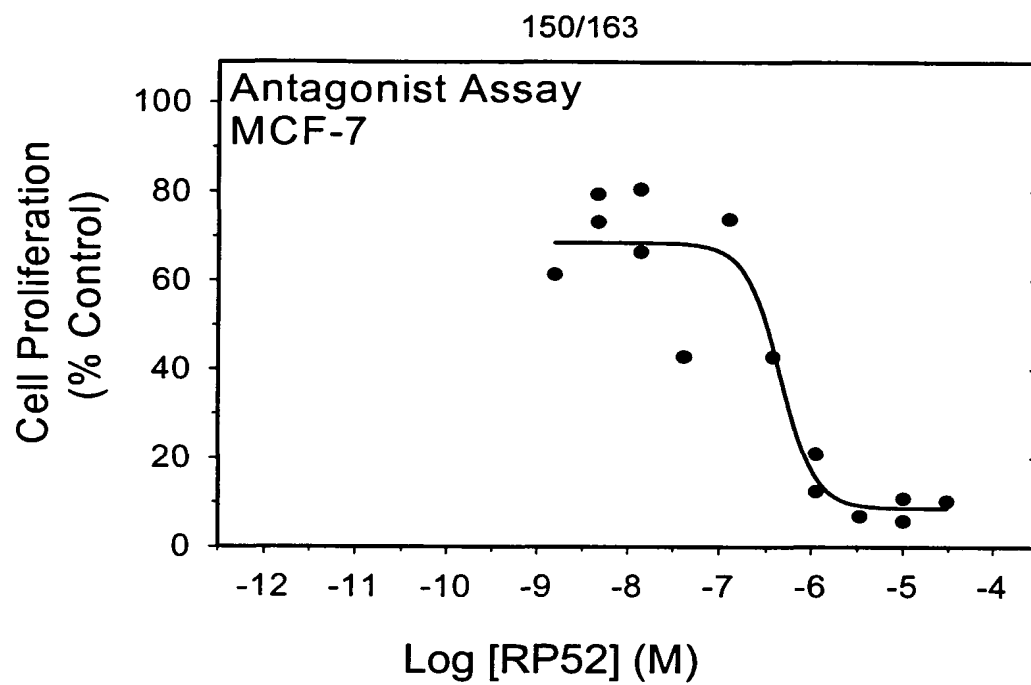
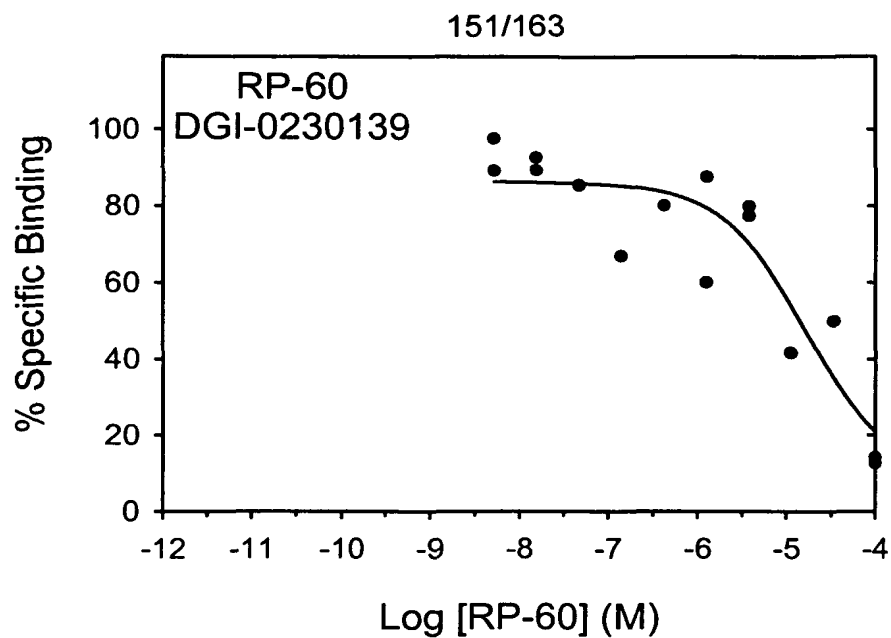
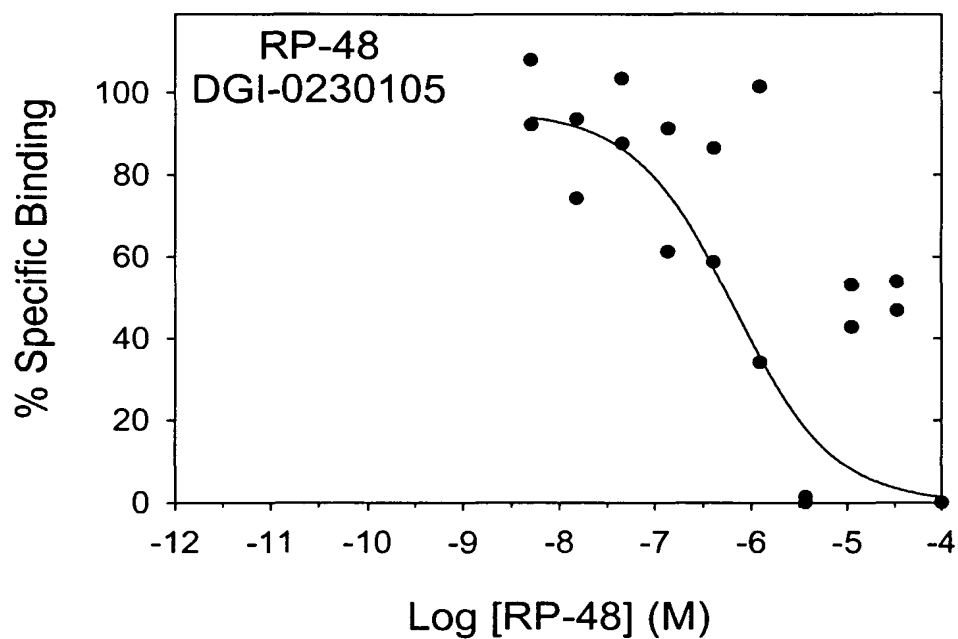
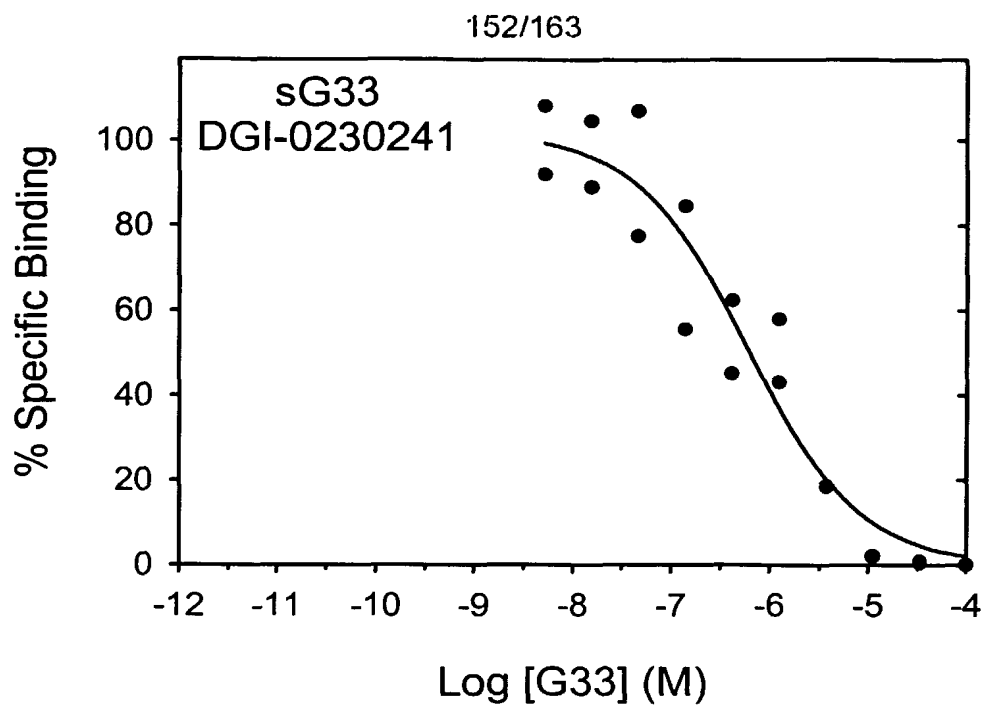
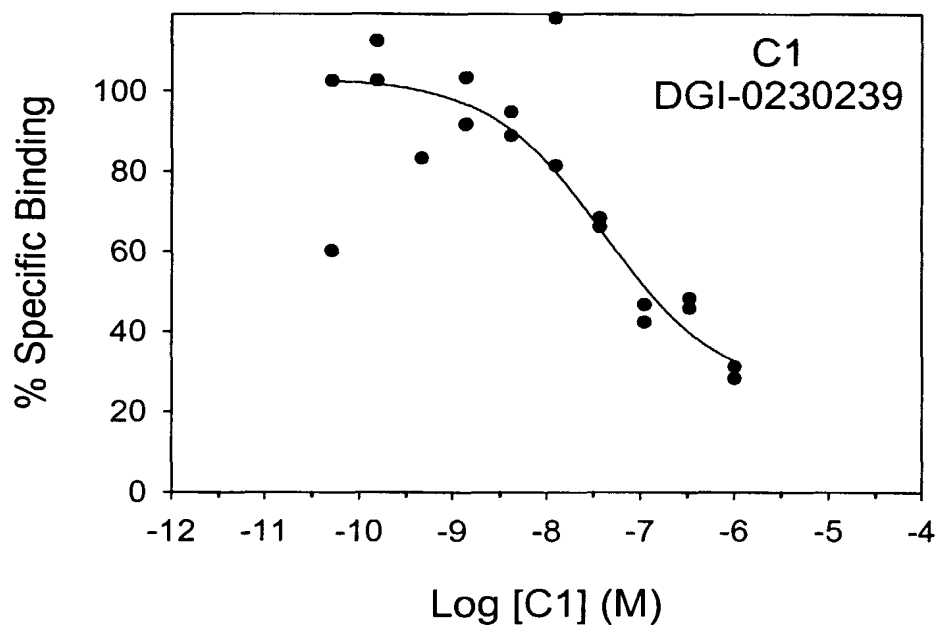


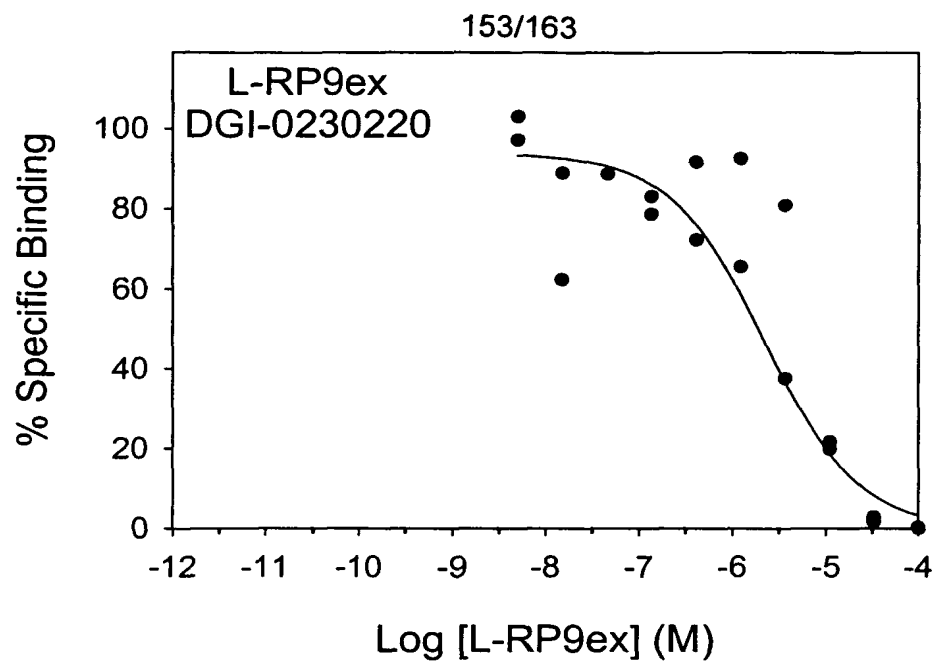
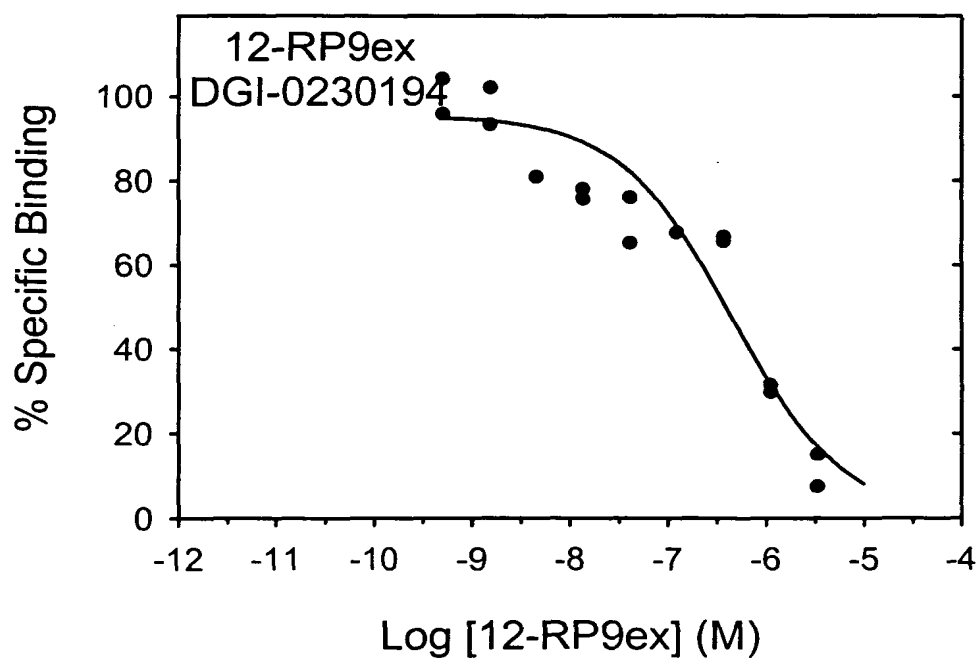
FIG. 69B

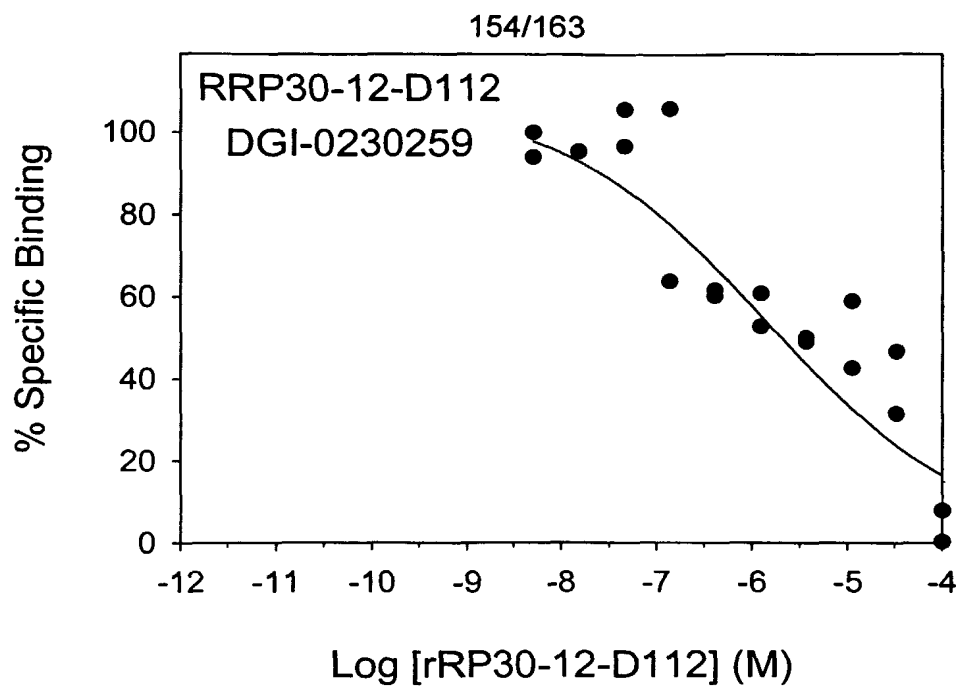
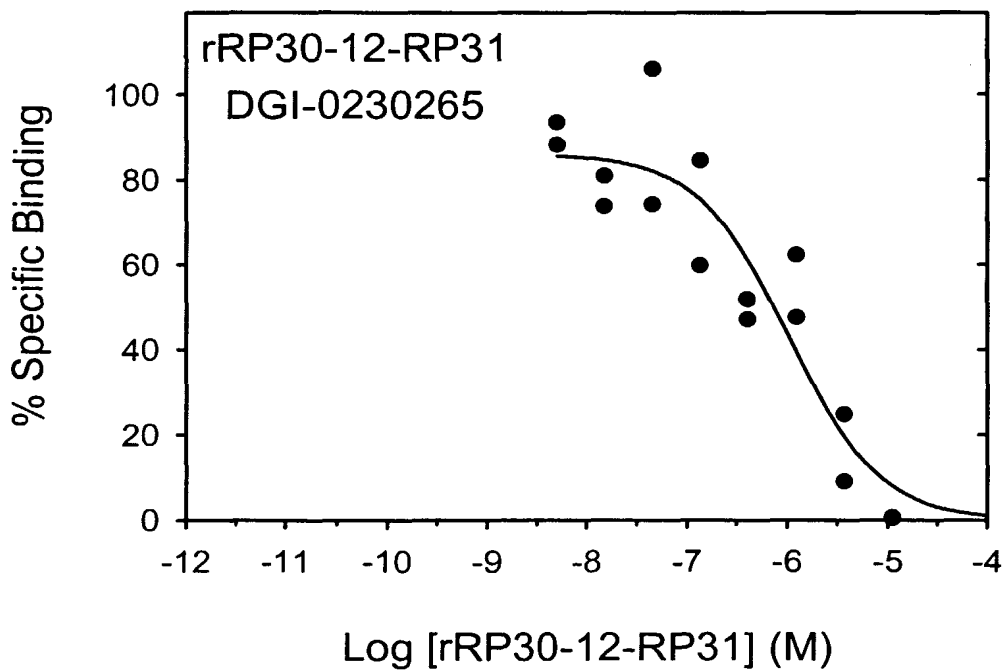
**FIG. 70A****FIG. 70B**

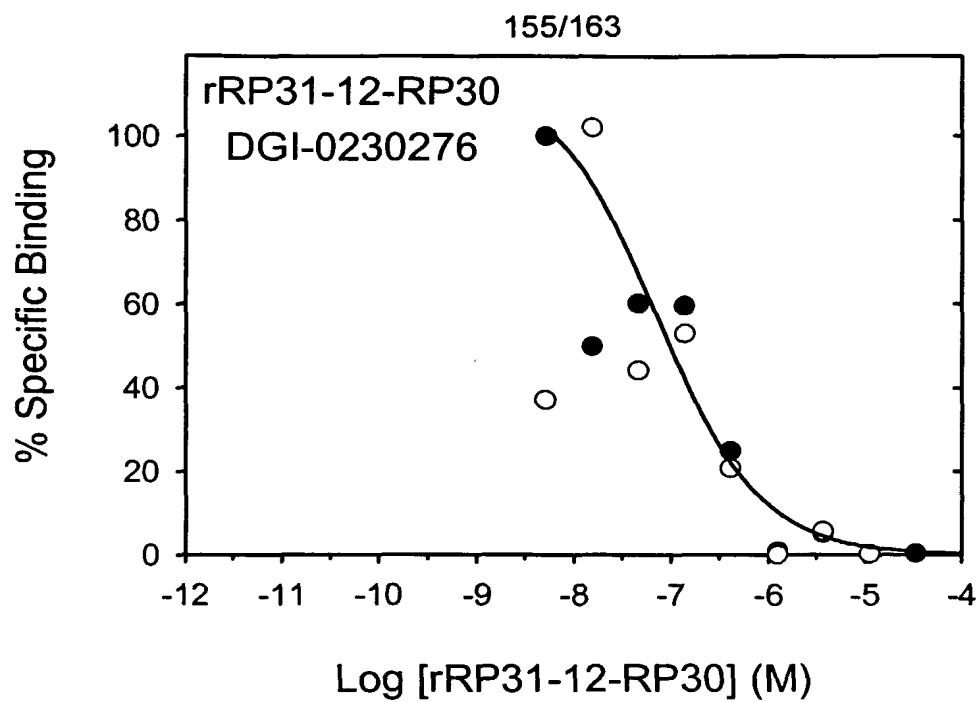
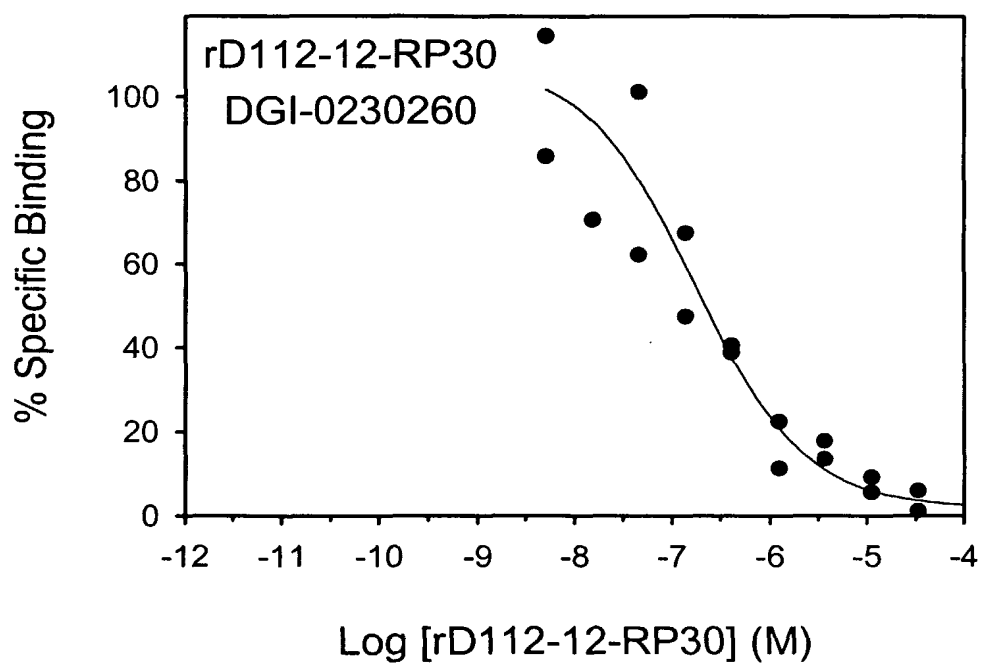
**FIG. 70C**

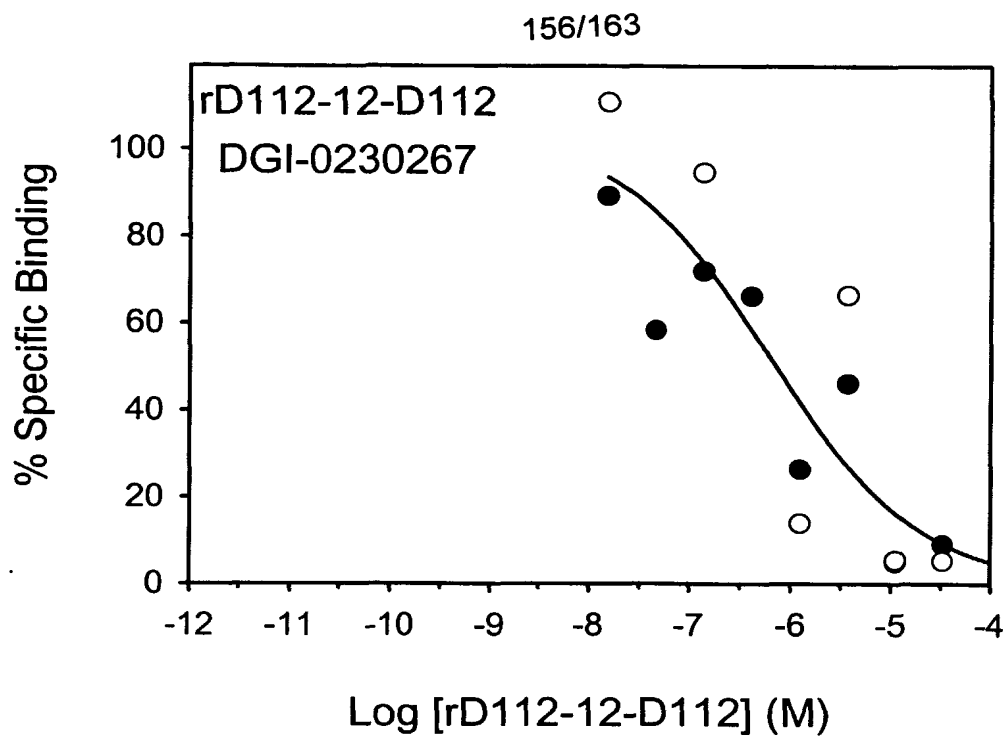
**FIG. 71A****FIG. 71B**

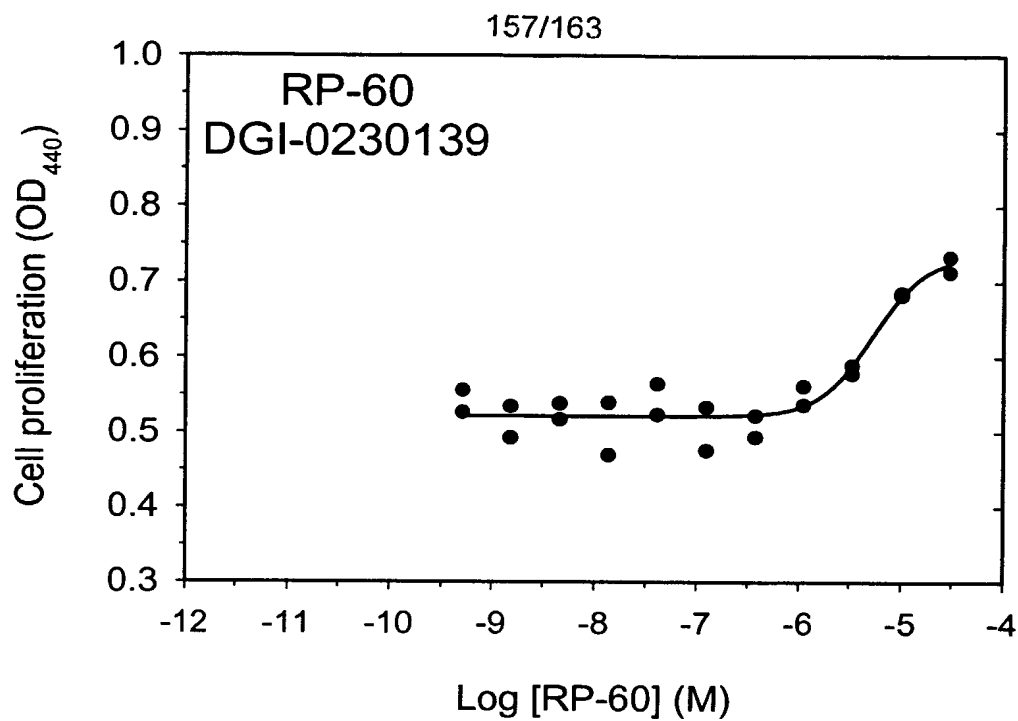
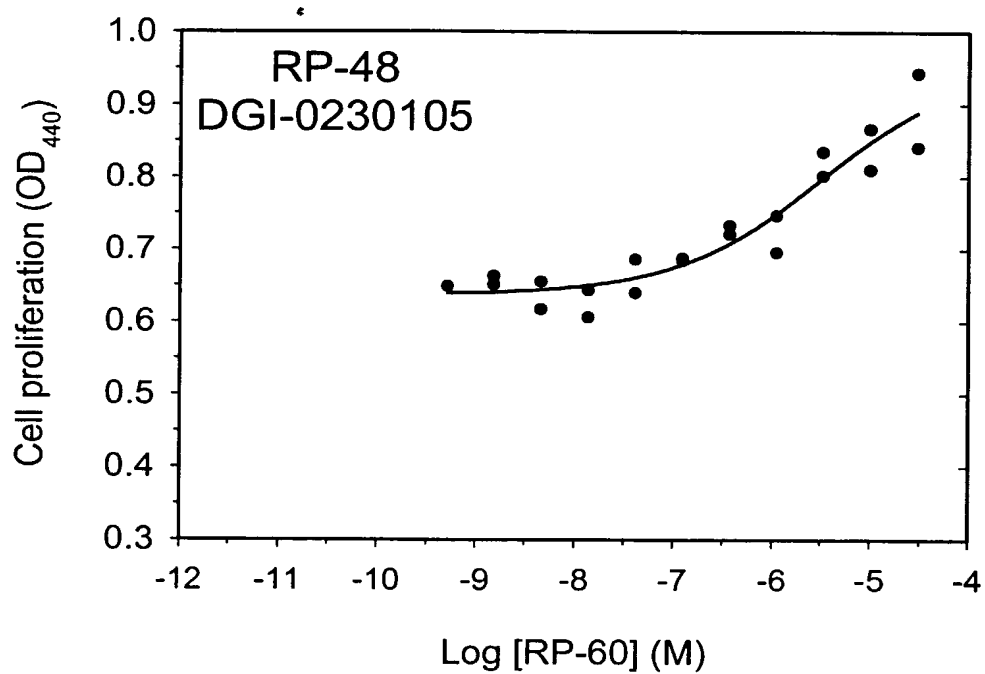
**FIG. 71C****FIG. 71D**

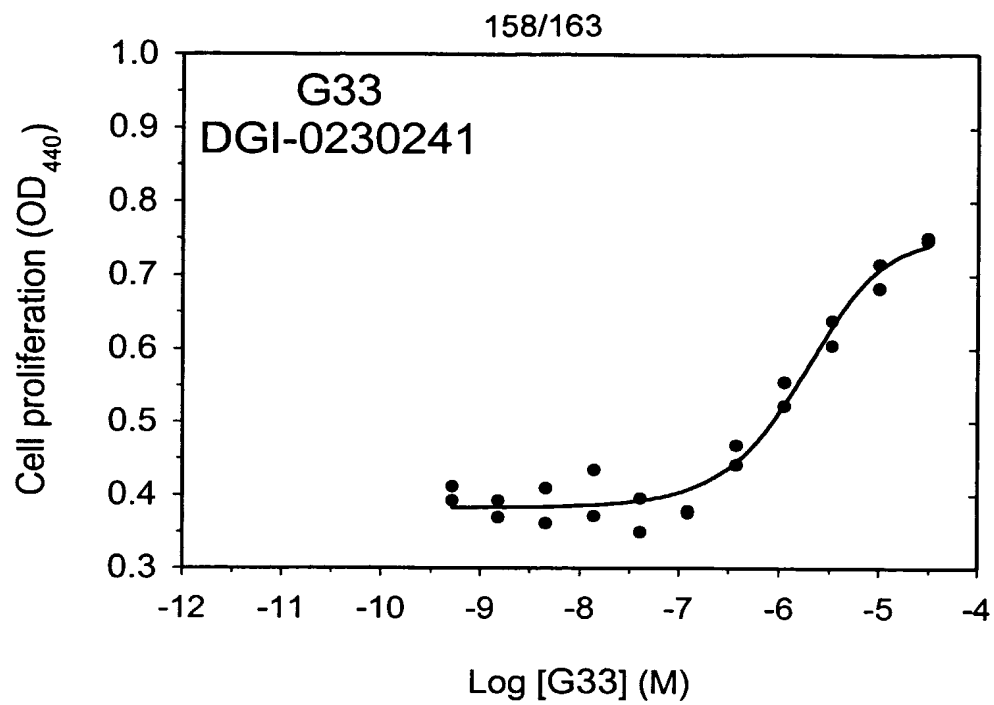
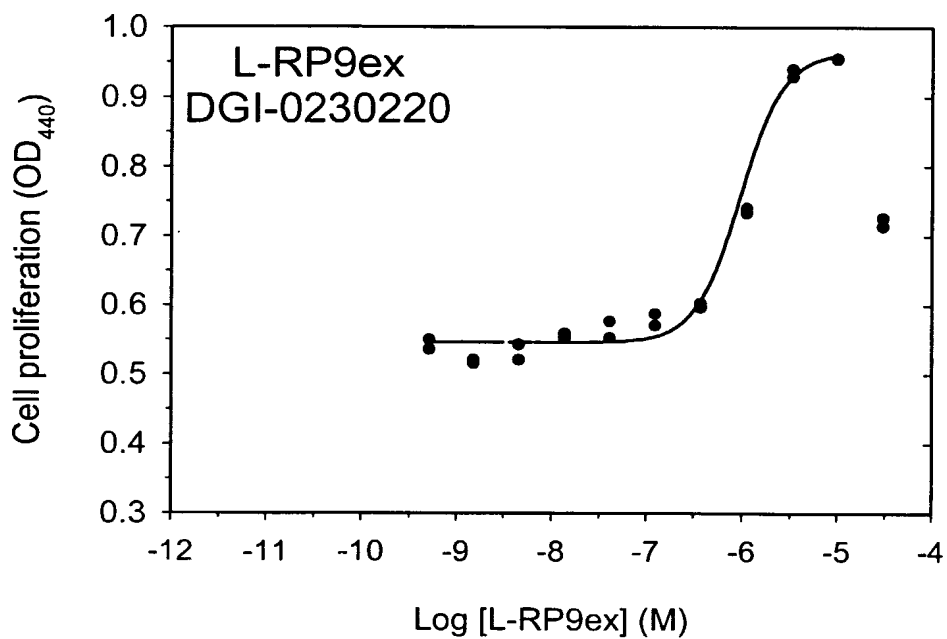
**FIG. 71E****FIG. 71F**

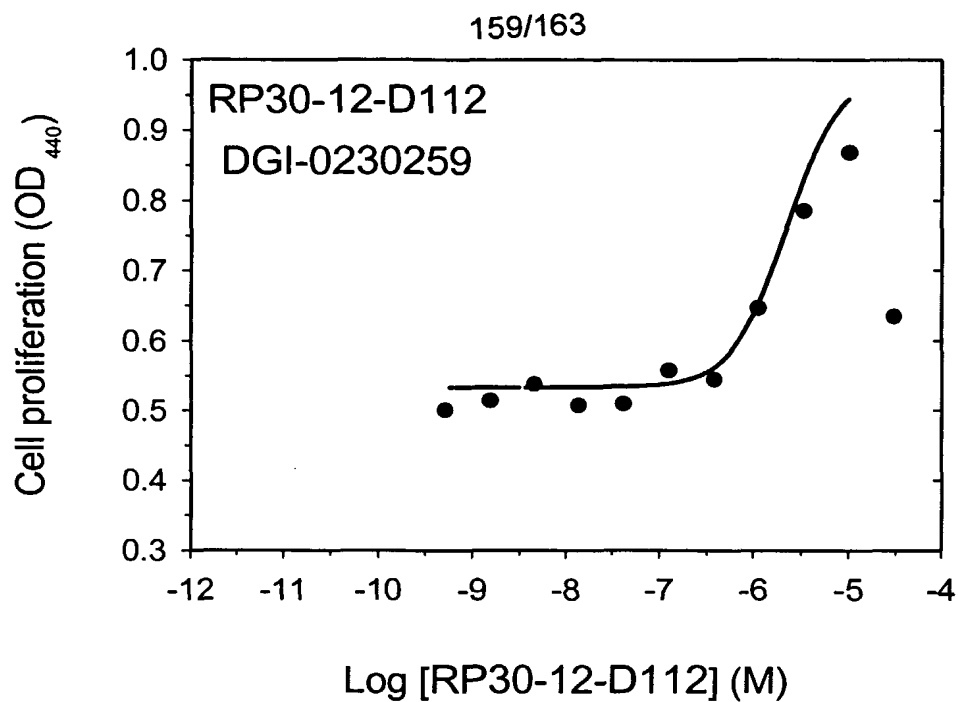
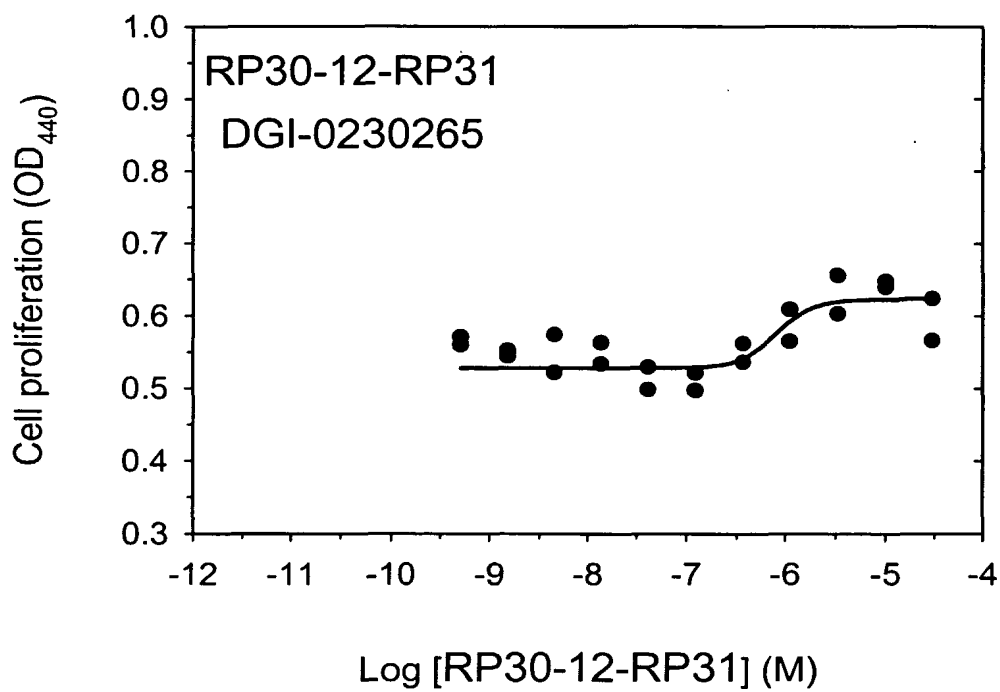
**FIG. 72A****FIG. 72B**

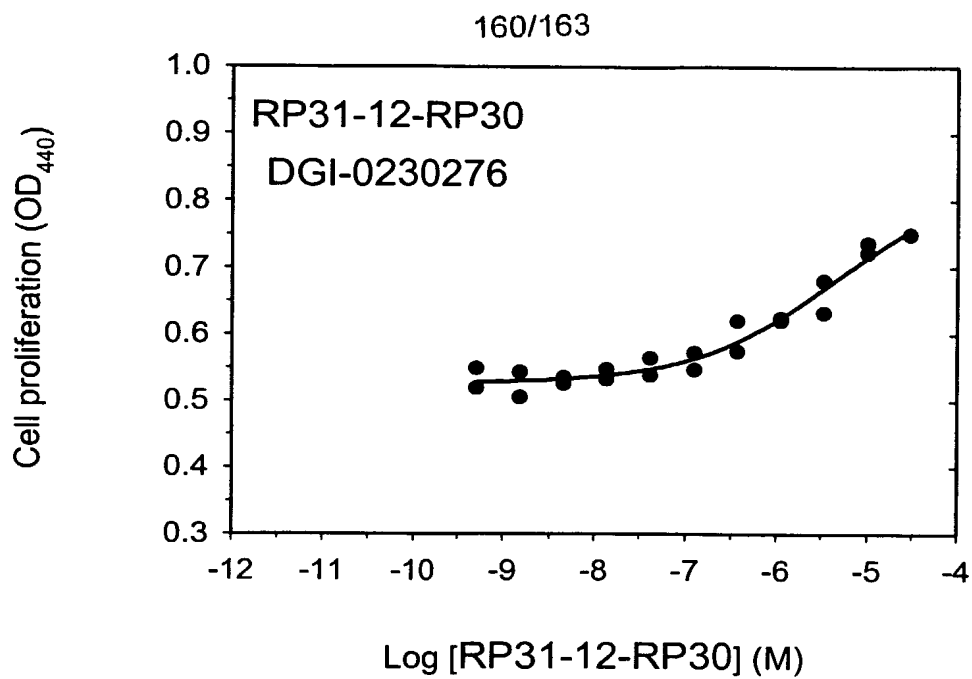
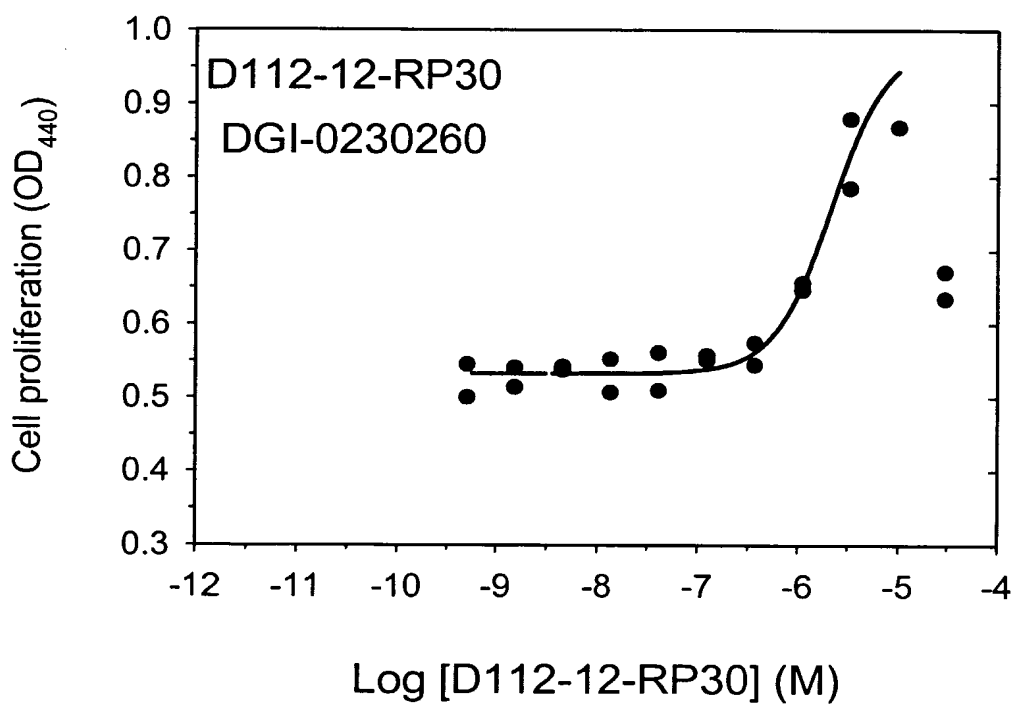
**FIG. 72C****FIG. 72D**

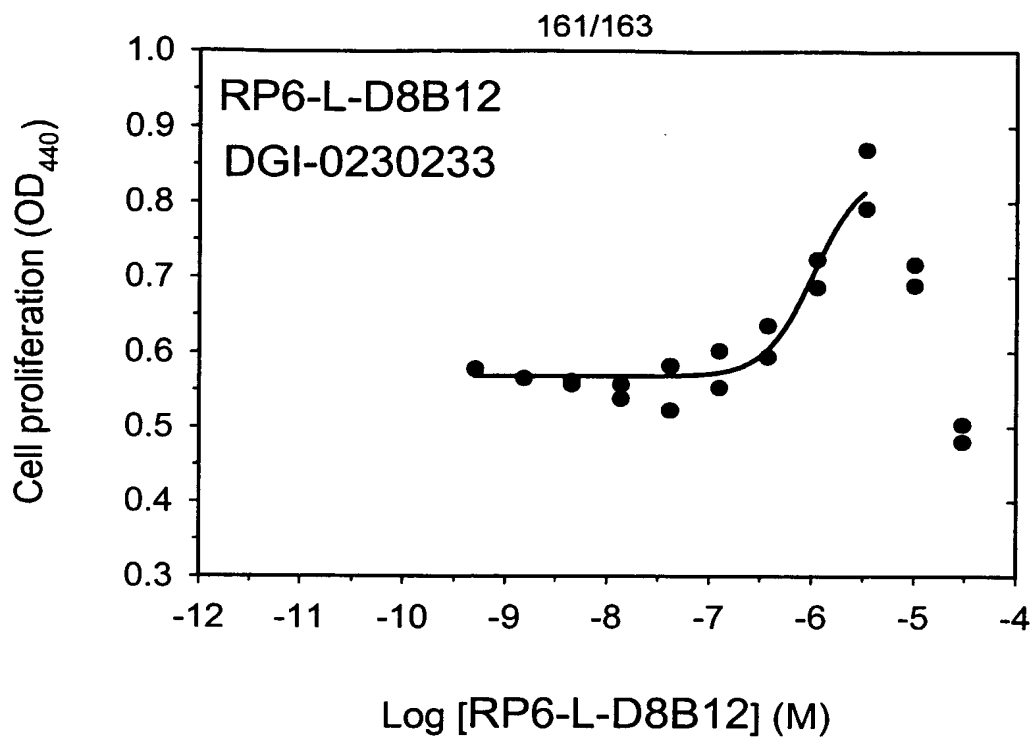
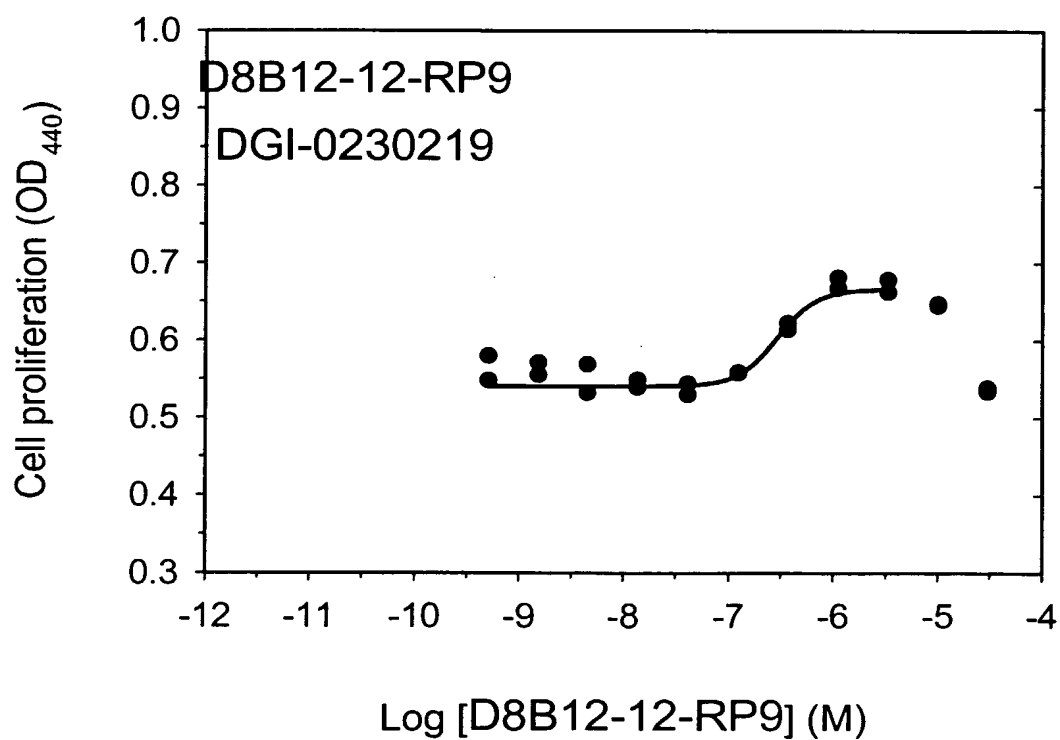
**FIG. 72E**

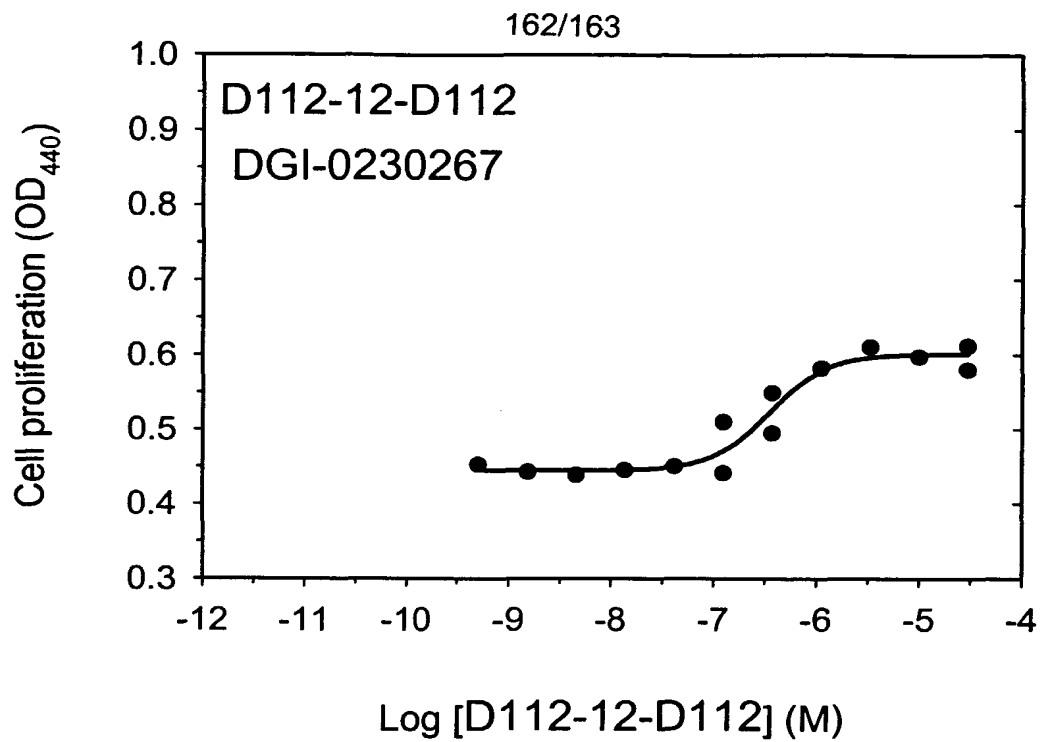
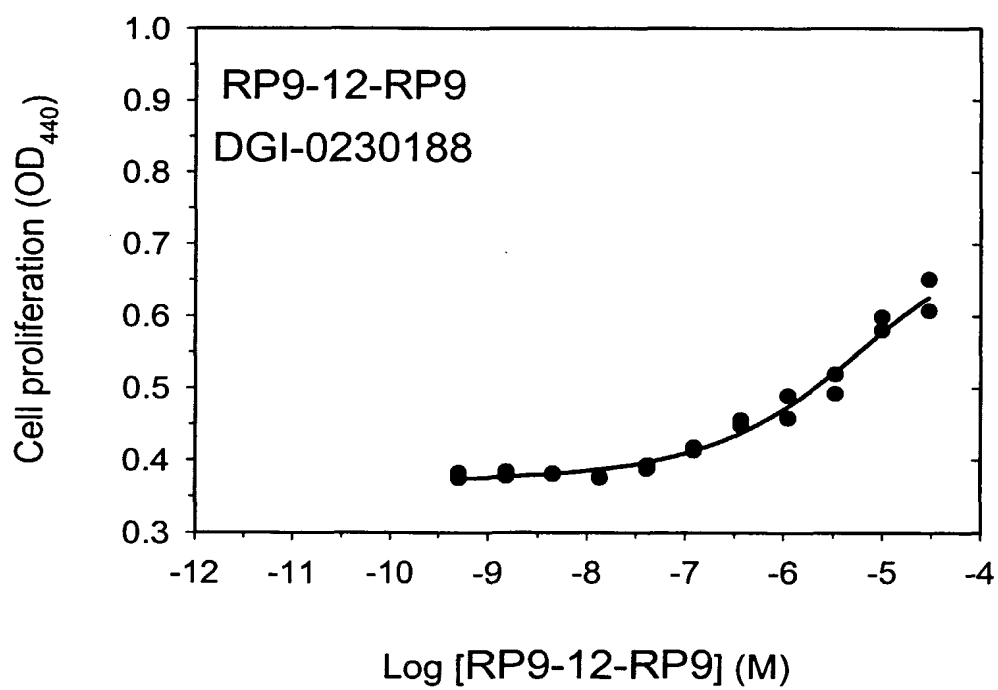
**FIG. 73A****FIG. 73B**

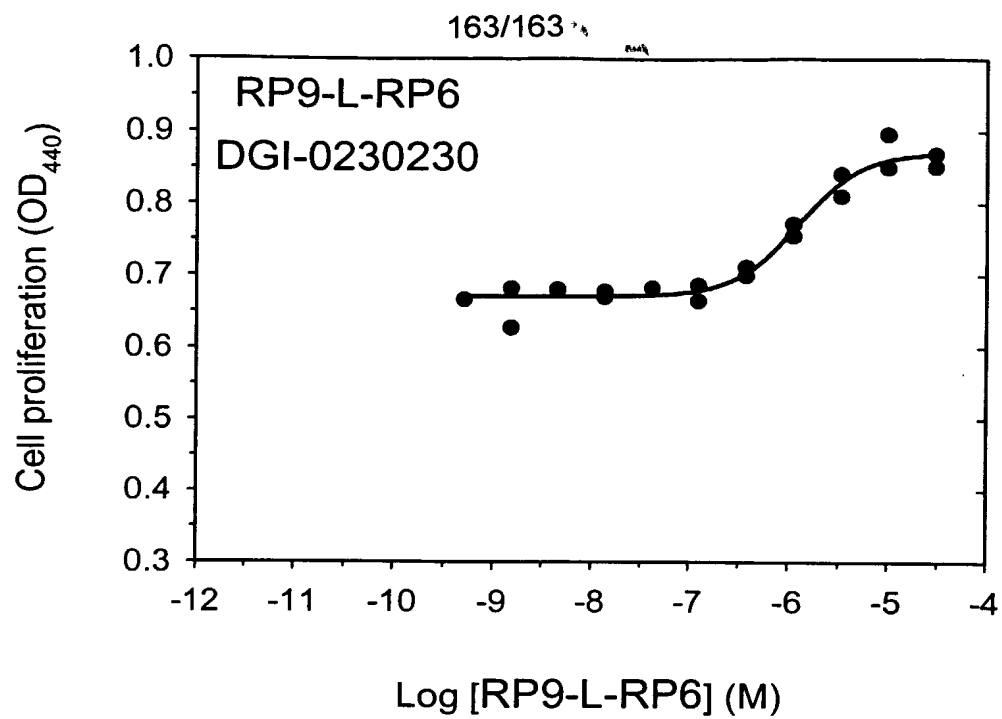
**FIG. 73C****FIG. 73D**

**FIG. 74A****FIG. 74B**

**FIG. 74C****FIG. 74D**

**FIG. 74E****FIG. 74F**

**FIG. 74G****FIG. 74H**

**FIG. 74I**